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FURTHER OBSERVATIONS ON THE NERVOUS SYSTEM OF THE AMERICAN LEOPARD FROG (RANA PIPIENS) COMPARED WITH THAT OF THE EUROPEAN FROGS (RANA ESCULENTA AND RANA TEMPORARIA)

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WITH TWO FIGURES

In a paper under the general title given above (Donaldson '08) I discussed some observations made in 1904 on R. esculenta at Zurich and R. temporaria at Liverpool.

On comparison with the American frog, R. pipiens, it was found that although the European species were very similar to the latter in form and proportions, nevertheless the weight of the central nervous system was significantly smaller in the European species, and in the case of R. esculenta, the number of medullated fibers in the spinal nerves was much less than in R. pipiens.

These observations made it possible to correct the records of Fubini ('81) on the weight of the brain and spinal cord, which had alone been available for the European forms, and to call attention to the possible bearing of the anatomical differences on physiological results obtained from the two European species on the one hand, and the American species on the other.

In view of the fact that on the basis of rather few observations I had ventured to designate Fubini's records as untrustworthy, and also to suggest possible physiological differences in the responses of the central nervous system, it seemed desirable to repeat the observations on the European forms.

This I did during the past summer. For a second time I am indebted to Professor Gaule for the hospitality of the Physiological Institute at Zurich, where I had examined R. esculenta in 1904, and to Professor Sherrington for similar privileges at the

Physiological Laboratory of University College at Liverpool, where I had examined R. temporaria in the same year.

To both these gentlemen I desire to express my obligations for their courtesy and aid.

The results of these latest observations support completely the conclusions based on the records of 1904.

In the present communication therefore it is not necessary to repeat the entire argument of the earlier paper, but merely to present the evidence for the similarity of the earlier and later records.

For this purpose it will be desirable to print in full only the original measurements for both years, while the important ratios can be given in condensed tables accompanied by a few charts.

The following are the tables of the principal measurements as made on the three species in 1904 and 1909

TABLE 1

Data on R. pipiens, Chicago 1904. 12 specimens

BODY WEIGHT IN GRMS.	SEX	TOTAL LENGTH IN MM.	BODY LENGTH IN MM.	WEIGHT IN GRAMS OF			PERCENTAGE OF WATER	
				C. N. S.	Brain	Sp. C.	Brain	Sp. C.
11.6	M.	130		.0918	.0666	.0252	84.4	79.4
16.0	M.	150		.1148	.0796	.0352	85.2	80.7
17.0	F.	159		.1054	.0714	.0340	84.0	80.6
20.8	M.	170		.1232	.0844	.0388	85.2	81.6
22.5	M.	162		.1165	.0807	.0358	84.5	80.4
26.4	M.	180		.1372	.0946	.0426	84.4	78.4
27.6	F.	179		.1416	.1014	.0402	84.8	80.1
30.6	M.	180		.1454	.0998	.0456	84.6	79.8
34.2	M.	190		.1518	.1056	.0462	85.6	81.6
41.8	M.	197		.1652	.1146	.0506	86.9	82.2
43.9	M.	200		.1708	.1210	.0498	85.8	80.7
47.0	M.	198		.1664	.1140	.0524	84.4	80.5

TABLE 2

Data on R. esculenta, Zurich 1904. 11 specimens

BODY WEIGHT IN GRMS.	SEX	TOTAL LENGTH IN MM.	BODY LENGTH IN MM.	WEIGHT IN GRAMS OF			PERCENTAGE OF WATER.	
				C. N. S.	Brain	Sp. C.	Brain	Sp. C.
12.40	F.	131		.0818	.0577	.0241	84.2	78.4
16.75	F.	144		.0926	.0634	.0292	83.4	79.1
18.43	F.	144		.0928	.0650	.0278	83.2	78.2
20.00	F.	161		.1103	.0756	.0347	82.5	79.2
22.00	.	164		.1107	.0769	.0338	84.0	79.0
24.10	M.	167		.1217	.0841	.0376	83.4	78.4
33.85	M.	175		.1327	.0895	.0432	83.2	78.2
36.30	M.	177		.1478	.1004	.0474	83.4	78.6
37.56	F.	188		.1490	.0993	.0497	82.9	78.8
37.96	F.	194		.1427	.0953	.0474	82.8	77.8
45.03	F.	196		.1578	.1078	.0500	83.9	78.4

TABLE 3

Data on R. esculenta, Zurich 1909. 11 specimens

BODY WEIGHT IN GRMS.	SEX	TOTAL LENGTH IN MM.	BODY LENGTH IN MM.	WEIGHT IN GRAMS OF			PERCENTAGE OF WATER	
				C. N. S.	Brain	Sp. C.	Brain	Sp. C.
18.9	M.	143	57.3	.1047	.0707	.0340	83.6	78.6
24.7	F.	167	65.0	.1065	.0719	.0346	83.6	79.3
26.5	M.	167	63.4	.1120	.0737	.0383	83.6	77.8
30.9	M.	177	69.2	.1198	.0830	.0368	83.9	78.5
32.3	F.	183	68.0	.1301	.0873	.0428	83.6	78.1
33.0	F.	184	70.5	.1275	.0845	.0430	83.4	77.0
35.5	F.	188	72.5	.1435	.0985	.0450	83.8	78.2
47.4	F.	204	80.0	.1593	.1063	.0530	83.1	79.4
48.4	F.	193	79.3	.1545	.1027	.0518	83.8	77.7
52.4	F.	205	82.0	.1589	.1105	.0484	83.6	79.1
58.0	F.	216	87.0	.1858	.1278	.0580	83.6	77.6

TABLE 4

Data on R. temporaria, Liverpool 1904. 12 specimens

BODY WEIGHT IN GRMS.	SEX	TOTAL LENGTH IN MM.	BODY LENGTH IN MM.	WEIGHT IN GRAMS OF			PERCENTAGE OF WATER	
				C. N. S.	Brain	Sp. C.	Brain	Sp. C.
14.05	F.	144		.0881	.0596	.0285	82.3	78.2
16.10	F.	151		.0991	.0690	.0301	82.7	79.0
17.65	M.	154		.0916	.0618	.0298	83.0	78.5
21.75	M.	171		.1045	.0671	.0374	82.8	78.2
23.45	M.	162		.0947	.0628	.0319	82.1	77.0
24.17	F.	173		.1333	.0864	.0469	81.9	76.5
27.05	M.	173		.1298	.0874	.0424	82.4	77.1
28.15	M.	168		.1018	.0687	.0331	82.5	76.7
28.95	M.	174		.1324	.0813	.0511	81.3	76.8
28.95	M.	178		.1485	.0928	.0557	81.3	76.8
32.15	M.	173		.1321	.0890	.0431	80.9	78.6
32.81	F.	178		.1161	.0766	.0396	82.7	78.0

TABLE 5

Data on R. temporaria, Liverpool 1909. 16 specimens

BODY WEIGHT IN GRMS.	SEX	TOTAL LENGTH IN MM.	BODY LENGTH IN MM.	WEIGHT IN GRAMS OF			PERCENTAGE OF WATER	
				C. N. S.	Brain	Sp. C.	Brain	Sp. C.
14.5	F.	148	53.3	.0772	.0522	.0250	82.2	76.8
17.2	M.	154	56.0	.0903	.0598	.0305	83.6	79.6
19.1	F.	155	58.5	.0907	.0621	.0286	82.4	76.3
21.0	M.	162	60.3	.1014	.0663	.0351	83.8	79.5
24.2	M.	176	64.5	.1099	.0736	.0363	83.9	78.5
25.4	M.	164	60.0	.0994	.0672	.0322	84.5	78.2
26.0	M.	162	60.8	.1066	.0702	.0364	84.0	79.4
26.1	F.	174	65.8	.1191	.0787	.0404	84.0	79.4
26.9	F.	163	63.2	.1092	.0737	.0355	84.1	76.3
27.9	F.	175	66.7	.1149	.0786	.0363	83.8	79.5
29.2	F.	174	66.5	.1114	.0744	.0370	83.8	79.5
29.4	M.	170	66.0	.1356	.0887	.0469	83.8	78.9
29.8	M.	168	62.5	.1167	.0751	.0416	83.8	78.9
32.1	F.	184	73.2	.1314	.0864	.0450	84.3	79.2
33.3	M.	167	64.0	.1373	.0900	.0473	84.3	79.2
39.1	F.	196	76.8	.1452	.0964	.0488	82.3	77.8

The foregoing tables (1-5), representing five series, contain the fundamental data.

The plan was to have twelve specimens in each series. In the case of R. esculenta 1904 and also 1909, there are, however, only eleven in each. The absent records were excluded because the percentage of water, which was not calculated until my return home, showed the excluded specimens to be in abnormal condition.

In the case of R. temporaria 1909 sixteen records were made. In general, the grouping of these data is by threes. There are however three exceptions: In R. esculenta 1904, with a total of 11 specimens, there is one group of two (Records 7 and 8) and in R. esculenta 1909, there is one group of two (Records 10 and 11).

In R. temporaria 1909 there is one group of four. In each case this departure from the rule is indicated in the condensed tables (6, 10, 12, 13,) by a bracketed number following the average for body weight.

It will be noted that in the 1904 series, the column under the heading "Body length" is vacant. This measurement was not made in that year, but was made in the specimens collected in 1909.

It represents the length of the frog from the tip of the nose to the tip of the urostyle, the skin over the urostyle having been split in order to expose its cartilaginous tip; the measurement being taken with vernier calipers.

In the previous paper (Donaldson '08) some measurements on preserved material were introduced without correction for the effects of the reagents used. These cases were explicitly noted. It is of interest to state therefore that, in this paper, the data apply to the fresh material only. Indeed all the measurements were made on the material when fresh except in the case of the leg bones of the two 1909 series. In these cases the legs were brought to this country from Europe in 60 per cent alcohol and then the bones were measured.

A long series of control observations on the legs of R. pipiens treated in the same way and for the same time have shown that

this treatment reduces the length of the femur by 0.70 per cent making it 99.30 per cent of the fresh length, the tibia by 0.73 per cent making it 99.27 per cent of the fresh length; the foot (tarsus-pes) by 1.54 per cent making it 98.46 per cent of the fresh length.

These corrections were applied before the data were used in tables 7 and 8.

TABLE 6

Body weight per millimeter of total length. Averages from groups of three

	BODY WEIGHT IN GRAMS	BODY WEIGHT PER MILLIMETER, IN GRAMS
R. pipiens	14.9	.102
	23.2	.135
	30.8	.168
	43.2	.218
R. esculenta 1904	15.9	.114
	22.0	.134
	35.0 [2]*	.199
	40.2	.208
R. esculenta 1909	23.4	.146
	32.1	.177
	43.8	.225
	55.2 [2]	.262
R. temporaria 1904	15.9	.107
	23.1	.137
	28.0	.162
	31.3	.177
R. temporaria 1909	18.0 [4]	.116
	25.2	.151
	26.9	.159
	29.5	.173
	34.8	.191

(A) AVERAGE AMOUNT OF BODY WEIGHT FOR EACH MILLIMETER OF TOTAL LENGTH

The general form of the specimens examined is obtained by dividing the body weight by the total length (table 6). The data in this table are given in Chart 1 and show that in the years

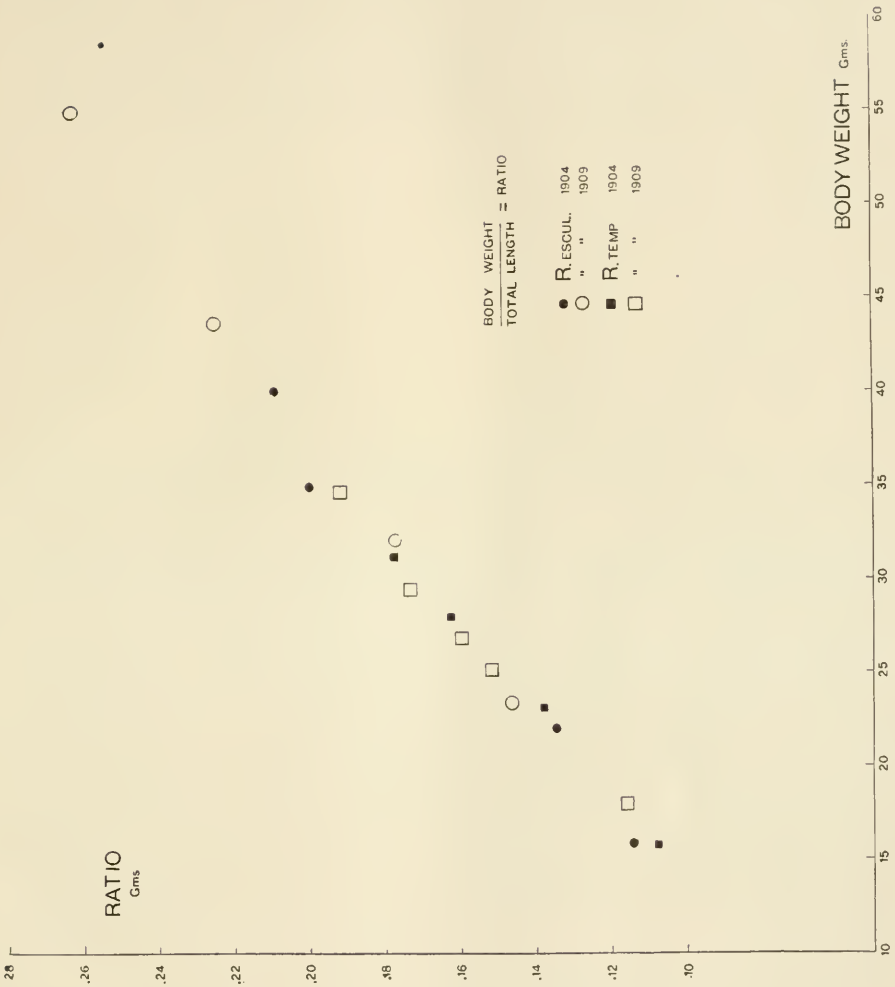

CHART I. Showing the average amount of body weight corresponding to each millimeter of the total length.

1904 and 1909, the European frogs were similar in their general form.

The records for R. pipiens are not entered on this chart. They would run a trifle below those for the European species, showing that R. pipiens was more slender in its general build. This character of R. pipiens taken alone would imply a slightly smaller nervous system, but as we know the contrary is the case.

TABLE 7

Percentage of the total length represented by the combined lengths of the leg bones

	SPECIMENS	PER CENT
R. pipiens	9	66.6
R. esculenta	11	65.1
R. temporaria	16	66.2

(B) PERCENTAGE OF TOTAL LENGTH REPRESENTED BY THE COMBINED LENGTHS OF THE LEG BONES

The absolute values of the percentages in this table are on the average less by 3.5 than those given in the previous paper (see Donaldson '08, table 2). This is the result of a change in the technique of measurement. Previously the total length of the frogs was taken when the animals were suspended, and under this condition a certain amount of flexion persisted in the legs.

In the present case the frog was measured when stretched out on the table and lying on its ventral surface. By this treatment the amount of flexion was reduced, and the total length thereby slightly increased. This naturally reduced the percentage value of the sum of the lengths of the leg bones, the measurements of which were made in the same way in both cases. The above mentioned change in technique is the only one which has been made.

The point of importance is that the percentages are nearly the same for the three species which are here compared.

(C) THE PROPORTIONAL LENGTHS OF THE SEVERAL LEG BONES

These are shown in table 8 in which the 1904 records have been repeated and a complete series of 1909 records added. It will be seen that there is no essential difference between the obser-

vations made at the contrasted dates, and that in both instances the proportional lengths are nearly the same for the three species compared.

TABLE 8

The proportional lengths of the several leg bones

	NO. OF SPECIMENS	FEMUR	TIBIA	FOOT (TARSUS AND PES)
		per cent	*per cent*	*per cent*
R. pipiens 1904	12	25.5	29.3	45.2
R. pipiens 1909	19	26.0	29.3	44.7
R. esculenta 1904*	5	26.3	28.2	45.5
R. esculenta 1909	12	26.8	28.2	45.0
R. temporaria 1904*	6	26.1	28.7	45.2
R. temporaria 1909	16	25.7	28.3	46.0

* Leg bones from frogs of the socalled "Zurich series of 1898." These frogs had been carefully fixed in 4% formaldehyde and then preserved in 80% alcohol. The effect of this on the lengths of the several leg bones was not at the time determined. (See Donaldson '08, p. 127).

(D) PERCENTAGE VALUE OF THE LENGTH OF THE ENTIRE CENTRAL NERVOUS SYSTEM—THE TOTAL LENGTH OF THE FROG BEING TAKEN AS THE STANDARD.

In the case of this character we have grouped the 1904 data (see Donaldson '08, table 5) into three entries and added the measurements on the new material for the 1909 groups.

The table shows that the length of the entire central nervous system is slightly greater in the European species. As this excess in length is associated with a deficiency in absolute weight, it follows, as was previously noted (Donaldson '08, p. 128) that the nervous system in R. pipiens must exceed that of the European species in its transverse diameters.

TABLE 9

Percentage value of the length of the entire central nervous system—the total length of the frog being taken as the standard

NO. OF SPECIMENS	AVERAGE TOTAL LENGTH IN MM.	PERCENTAGE VALUE OF THE LENGTH OF THE ENTIRE CENTRAL NERVOUS SYSTEM		
		Rana pipiens	Rana esculenta	Rana temporaria
9 (1904)	152	17.5		
4	155			17.6
3	159		18.4	
3	167			17.6
6	171			17.3
9 (1904)	176	16.7		
3	181		16.9	
3	182			17.2
3	195		16.6	
4 (1904)	196	16.3		
2	210		16.2	

(E) THE WEIGHT OF THE CENTRAL NERVOUS SYSTEM

Turning now to the main character under consideration, the weight of the central nervous system, the condensed records are presented in table 10.

When these data are put in the form of a chart, (chart 2) several interesting relations between the observations of 1904 and those of 1909 at once appear. In the first place the later records follow the same line as the earlier; second, the record for each species in 1909 is somewhat less than in 1904, and as a consequence still further below the records of 1904 for R. pipiens. This result serves to establish the main conclusion, namely that R. pipiens has a heavier nervous system than either of the European forms. The fact that the values for the weight of the central nervous system in the European species as determined in 1909 are less than those determined in 1904, calls for a word of comment.

Some unpublished studies which are being made on R. pipiens at the Wistar Institute relative to the change in the weight of the central nervous system with season, indicate that in this species the greatest weight is attained about the end of July.

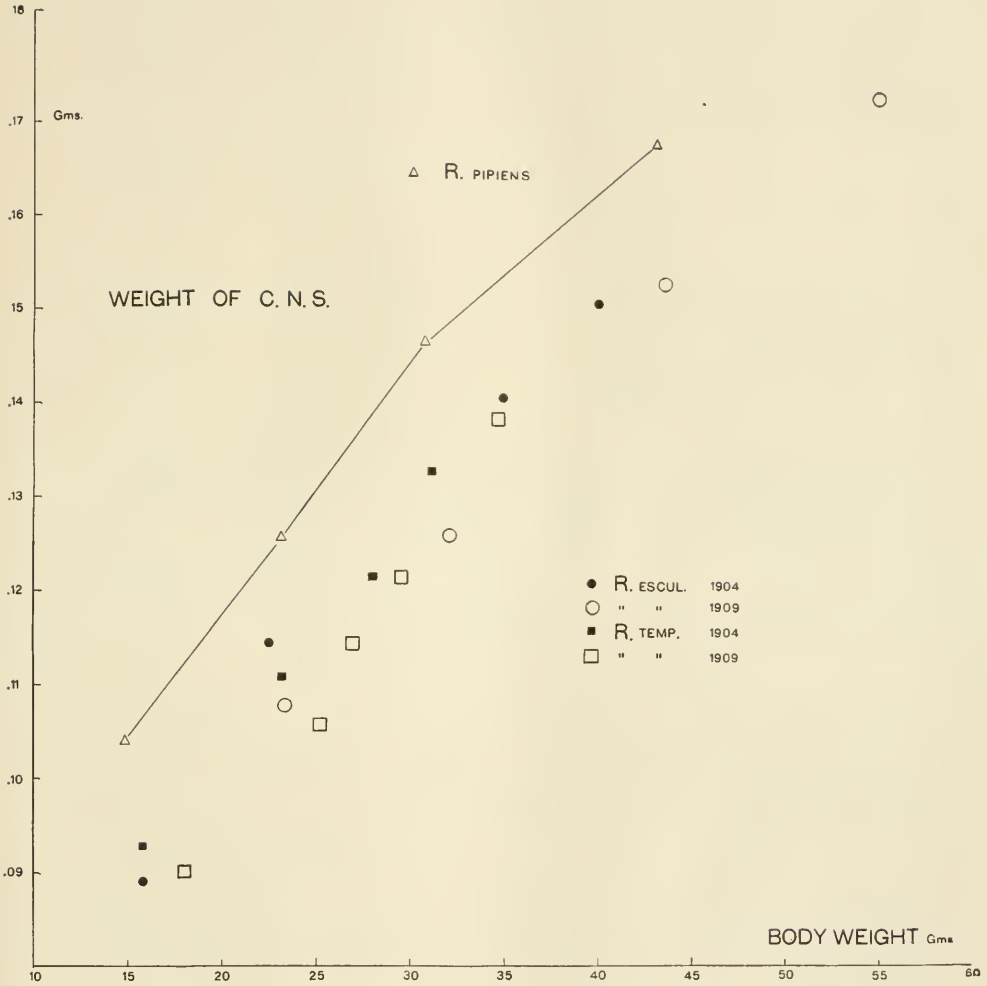

CHART 2. Showing the weight of the entire central nervous system.

TABLE 10

Weight of the central nervous system in grams. Averages from groups of three

	BODY WEIGHT	WEIGHT OF CENTRAL NERVOUS SYSTEM
R. pipiens	14.9	.1040
	23.2	.1256
	30.8	.1463
	43.2	.1674
R. esculenta 1904	15.9	.0890
	22.0	.1142
	35.0 [2]	.1402
	40.2	.1498
R. esculenta 1909	23.4	.1077
	32.1	.1258
	43.8	.1524
	55.2 [2]	.1724
R. temporaria 1904	15.9	.0929
	23.1	.1108
	28.0	.1213
	31.3	.1323
R. temporaria 1909	18.0 [4]	.0899
	25.2	.1053
	26.9	.1144
	29.5	.1212
	34.8	.1380

If this observation applies, as it probably does, to the European species, then the differences in weight as shown in chart 2 are susceptible of the following explanation:

The esculenta of 1904 were examined August 1—5, when it may be assumed that the nervous system of R. esculenta had attained approximately its maximal seasonal weight. In 1909 the examination was from July 5-7, or some four weeks earlier. Under these circumstances, a somewhat smaller weight was to be expected, and the records show this.

The temporaria of 1904 were examined July 11 and 12, before the central nervous system had reached the maximum for the season.

In 1909 the examination was from August 17 to 21, or some

three weeks after the assumed maximum, and at a time when the seasonal weight has begun to diminish. Here the difference is less than in the case of the esculenta, but is susceptible of a similar explanation.

The relation of these two series of observations can be conveniently shown in still another way.

I have been able to point out (Donalsdon '02) that a fairly accuate determination of the weight of the central nervous system in frogs can be made by the formula

$$C.\ N.\ S. = (Log.\ Bd.\ W. \sqrt[4]{L})\, C$$

where *C. N. S.* is the weight of the central nervous system, *Bd. W.* the body weight in grams, *L* the total length in mm. and *C.* a constant to be determined for each species. Since publishing this formula I have found that the most convenient way of expressing seasonal variations on the weight of the central nervous system is by the variations in *C.*

Applying this method to the series before us, and remembering that the increase in the relative weight of the central nervous system is measured by the increase in *C*, and vice versa, we obtain the following:

TABLE 11

To show the values of "C" for each of the several series

	AVERAGE BODY WEIGHT	VALUE OF C.
R. pipiens 1904 Average of 12 records	28.0	26.2
R. esculenta 1904 Average of 8 records First "weight group" omitted	32.4	24.6
R. esculenta 1909 Average of 9 records Last "weight group" omitted	33.1	23.0
		Difference 1.6
R. temporaria 1904 Average of 12 records	24.6	22.8
R. temporaria 1909 Average of 16 records	26.8	21.9
		Difference 0.9

As is to be seen by inspection of the foregoing table 11 the value of *C* for the 1904 records is greater in both the European species than for the corresponding 1909 records, and as noted above, the greatest difference (1.6) is in the case of R. esculenta.

In connection with this table a word of explanation is required. It has been found that there is a slight increase in the value of *C* as the absolute size of the frog increases. This is a relation previously overlooked, but which will be discussed elsewhere. The bearing of it on the present case is that in making a comparison of the values of *C* in any pair of records, it is necessary in order to get trustworthy results, to compare the determinations for frogs of approximately the same range in size. In the present instance this makes it necessary in the case of R. esculenta to omit the value of *C* for the first weight group of the 1904 series, because there is no corresponding weight group on the 1909 series, and similarly to omit the determinations for the last weight group of the 1909 series.

A glance at chart 2 will serve to supplement the explanation.

In the case of the records for R. temporaria, the values for *C* in all the weight groups of both years have been used in making up the averages. It is because of this influence of the absolute size that the average body weights for each series are entered in table 11.

All through the present paper the data on R. pipiens used in 1904 have been repeated without revision. In the former communication (Donaldson '08. pp. 132-133) it was noted that the weight of the central nervous system in the series of this species was low in comparison with other data which we had. This statement still holds good, but it was thought wiser to leave the standard as represented by 1904 records on R. pipiens unchanged at this time.

As evidence that the weights here used were low for this species, I give below two other series of determinations of *C* on Chicago frogs as follows:

NUMBER OF SPECIMENS	DATE ABOUT AUG. 1	AVERAGE BODY WEIGHT GMS.	AVERAGE VALUE FOR C.
48	1902	22.3	28.6
4	1909	27.7	29.5

It will be seen on comparison with the value of C. for the series of R. pipiens here used ($C = 26.2$) that these are much higher. This implies an increase in the weight of the central nervous system proportional to the differences in the values of C after correction for the differences in body weights in the several series.

Why the particular series of pipiens used by me as a standard falls below that for the two other series is a point the discussion of which must be reserved for a future paper.

In this connection it is desirable to refer to one modifying condition affecting the value of C which has not heretofore been mentioned, and the data on which are still unpublished. I find that the value of C is not the same for specimens of R. pipiens from different parts of our own country. For example those coming from northern Minnesota give a value sensibly greater than that found for the socalled "Chicago frogs" and the specimens taken about Philadelphia give a value less than that found for the "Chicago frogs," as a rule, but almost identical with that of the series used as a standard in this paper.

R. pipiens extends much farther south in this country of course, being found both in Florida and Texas. What the relation of C may be in specimens from stations farther south than Pennsylvania, has still to be determined, but the possibility of variation in this character with latitude is a matter of much interest.

(F) THE RATIO OF THE WEIGHT OF THE BRAIN TO THAT OF THE SPINAL CORD

Omitting the tabulation of the absolute values for the brain and cord, as these can be readily found in the full tables, I give below in table 12 a condensed statement of the ratios.

It will be seen that in both 1904 and 1909, that relative weight of brain (the value given under "ratio") is higher in R. esculenta than in R. temporaria, although the difference is not so great in the later as in the earlier records. Further, this ratio in R. pipiens is always greater than in either of the European forms.

Finally it is to be noted that the ratios which I find for the

European species are much higher than those determined by Fubini (see Donaldson '08, table 20) and so confirm my earlier conclusions concerning the untrustworthy character of his records.

TABLE 12

Ratios of the weight of the brain to the weight of the spinal cord. Averages from groups of three

	BODY WEIGHT	RATIO
R. pipiens	14.9	2.33
	23.2	2.22
	30.8	2.32
	43.2	2.28
R. esculenta 1904	15.9	2.29
	22.0	2.22
	35.0 [2]	2.09
	40.2	2.05
R. esculenta 1909	23.4	2.03
	32.1	2.09
	43.8	2.05
	55.2 [2]	2.24
R. temporaria 1904	15.9	2.15
	23.1	1.86
	28.0	1.87
	31.3	1.87
R. temporaria 1909	18.0 [4]	2.02
	25.2	2.02
	26.9	2.06
	29.5	1.90
	34.8	1.93

(G) THE PERCENTAGE OF WATER IN THE BRAIN AND SPINAL CORD

Table 13 shows the condensed results on the percentage of water. In my former communication I called attention to the differences in this character in the several species (Donaldson '08, p. 139.)

While the percentages of water in both the brain and spinal cord as determined for both European species in 1909 are less than that found in R. pipiens, they are nearly alike, and also similar to the 1904 determination for R. esculenta, so that it is

not desirable to give any weight to the differences as observed in 1904.

The value of this table as it stands is to show that we were dealing in all cases with healthy frogs, as the frog readily shows by changes in the amount of water in the nervous system, the effect of infections or disturbing conditions.

TABLE 13

Showing the percentage of water in the brain and in the spinal cord. Averages from groups of three

	BODY WEIGHT	PERCENTAGE OF WATER IN Brain	Spinal Cord
R. pipiens	14.9	84.5	80.2
	23.2	84.7	80.1
	30.8	85.0	80.5
	43.2	85.7	81.2
R. esculenta 1904	15.9	83.6	78.6
	22.0	83.3	78.9
	35.0 [2]	83.3	78.4
	40.2	83.2	78.3
R. esculenta 1909	23.4	83.6	78.6
	32.1	83.6	77.9
	43.8	83.6	78.4
	55.2 [2]	83.6	78.3
R. temporaria 1904	15.9	82.7	78.6
	23.1	82.3	77.2
	28.0	82.1	76.9
	31.3	81.6	77.8
R. temporaria 1909	18.0 [4]	83.0	78.1
	25.2	84.1	78.7
	26.9	84.0	78.4
	29.5	83.8	79.1
	34.8	83.6	78.7

The foregoing tables and the comments on them are intended to demonstrate that a second series of observations on R. esculenta and R. temporaria made in 1909 at an interval of five years yield results substantially similar to those first obtained in 1904

and that therefore so far as the conclusions of the earlier paper depend on the observations which have been repeated, they may be considered as well founded.

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1881

ON THE LENGTH OF THE INTERNODES IN THE SCIATIC NERVE OF RANA TEMPORARIA (FUSCA) AND RANA PIPIENS: BEING A RE-EXAMINATION BY BIOMETRIC METHODS OF THE DATA STUDIED BY BOYCOTT ('04) AND TAKAHASHI ('08)

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WITH THREE FIGURES

In 1904 Boycott published an important paper on the length of the internodes under the title "On the number of nodes of Ranvier in different stages of the growth of nerve fibers in the frog." In this work he measured 5338 internodes from twenty-six frogs which ranged in body length from 14.5 mm. to 53.9 mm. Although his observations were limited to the fibers from the thigh taken at the division of the sciatic nerve into the n. tibialis and n. peroneus, they nevertheless give us definite information as to the lengthening of the internodes first in relation to the lengthening of the leg, and second in relation to the increase of the fibers in diameter.

Four years later (1908) Takahashi published a paper on the same subject under the title "Some conditions which determine the length of the internodes found on the nerve fibers of the leopard frog, Rana pipiens," and in this investigation he examined several new points. Takahashi's observations were made not only at three different levels in the thigh, but also at levels in the shank and in the foot, as well as on some of the spinal nerve roots.

He measured altogether 3068 internodes from eight frogs mostly of a larger size than those used by Boycott. The body lengths of Takahashi's specimens ranged from 39.0 mm. to 89.4 mm.

The examination of the two interesting papers just mentioned, suggested several points for further study. It seemed desirable to determine first, how far the results obtained by the simple method of averages would agree with those obtained by the more elaborate statistical treatment of the data; and second, whether or not there is some definite law expressing the relation between the length of the internode and diameter of the fiber on which it occurs, both in different segments of the leg of the same frog, as well as on the same segment of the leg in frogs of different sizes.

In order to investigate the problems just mentioned, it was necessary to reëxamine the original data used in the foregoing researches, and through the efforts of Prof. H. H. Donaldson, I was so fortunate as to get the generous permission of both Boycott and Takahashi to use their valuable original data for this investigation, and I wish to express here my thanks to all of these gentlemen.

METHOD OF TREATING THE DATA

As has been shown by Boycott and Takahashi, the length of the internode is highly variable, even on the same fiber, as well as on fibers of the same diameter, and therefore in order to get proper mean values in the case of Boycott's records, I have combined the data for several frogs, and out of the total of twenty-five frogs, made five groups according to the length of the sciatic nerve as determined by Boycott.[1]

Group 1. Those with a sciatic nerve measuring from 15.5 mm. to 17.0 mm.: represented by 2 frogs.

Group 2. Those with a sciatic nerve measuring from 20.0 mm. to 22.5 mm.: represented by 7 frogs.

Group 3. Those with a sciatic nerve measuring from 24.0 mm. to 26.5 mm.: represented by 6 frogs.

[1] The length of the sciatic nerve as defined by Boycott is the distance from the point of emergence from the vertebræ of the upper of the two larger branches of the plexus to the level of the nerve obtained by cutting across the leg through the knee joint when it is in full extension; *loc. cit.*, p. 371.

Group 4. Those with a sciatic nerve measuring from 30.5 to 37.0 mm.: represented by 4 frogs.

Group 5. Those with a sciatic nerve measuring from 46.0 mm. to 53.5 mm.: represented by 6 frogs.

In the case of Takahashi's data, I found that the internodes had been measured at the lower end of the thigh in only four frogs, giving a total of 683 measurements. These frogs moreover differ so widely in body weight that the data are insufficient for biometric treatment. For this reason I have decided to analyze Boycott's data as completely as possible and then merely to compare the results obtained with Takahashi's conclusions.

I shall present my results in the following order:

I. A confirmation of Boycott's and Takahashi's conclusions together with my own.

II. Analysis of Boycott's data.

a. Analytical constants.

b. Frequency distributions.

c. Mean and standard deviation.

d. On the correlation in growth between internodal length and the diameter of the fiber.

III. Takahashi's observation on the length of the internode in different segments of the same frog.

IV. Correlation tables.

I. As my principal object was to find whether there is any definite law relating to the length of the internode and its diameter in different segments of the leg from the same frog, as well as in the same segment of the leg in different sized frogs, I shall not touch many other points discussed by Boycott and especially by Takahashi. Within the range of my examination, I confirm all their findings. Since the evidence of such confirmation will be found in the following pages, I shall present here only the main facts brought out by myself.

1. In a given specimen of Rana temporaria (fusca) whatever its size, the length of an internode and its diameter are positively correlated, though the correlation is not high; therefore it can be stated that the internodal length varies as the diameter. The degree of correlation increases as the frog becomes larger.

2. When however the lengths of the internodes for given diameters in different sized frogs are compared, the larger frog has for a given diameter longer internodes than the smaller frog. Thus in this case the internode varies according to the relation given by the following general exponential equation

$$y = Ae^{hx}$$

where the constants A and h are to be determined from the observations, y is the internodal length, x the diameter and e the base of the natural system of logarithms.

3. The equation just mentioned expresses also the relation in these two characters in the different segments of the leg from the same frog.

4. Therefore *the rate of the increment of the length of the internode following the increase in the diameter is proportional to the length of the internode itself*, or in mathematical terms.

$$\frac{dy}{dx} = hy$$

This may be considered as the general formula which expresses the relation of the two characters in different segments of the leg of the same frog as well as in corresponding segments from frogs of different sizes.

II. Analysis of Boycott's Data

a. Analytical Constants

For future reference I shall present here the various values of the analytical constants as determined from Boycott's data.

TABLE 1

Showing the values in terms of micra of the analytical constants determined from Boycott's data on Rana temporaria (fusca)

		GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V
No. of measurements		157	1097	1515	605	1916
μ_2	Diameter	1.7234	1.7785	2.2991	3.3327	3.3265
	Internode	1.9359	2.4773	2.6121	5.2862	12.3402
μ_3	Diameter	−1.1326	−0.1980	−1.5720	−2.6559	−2.7581
	Internode	1.2894	3.5331	5.0630	9.4191	30.3224
μ_4	Diameter	9.7848	8.0596	14.3649	43.5208	32.5211
	Internode	19.3369	29.6266	60.1877	110.6646	585.2832
β_1	Diameter	0.2506	0.0070	0.2034	0.1906	0.2067
	Internode	0.2289	0.8212	1.4383	0.6006	0.4893
β_2	Diameter	3.2944	2.5481	2.7176	3.9184	2.9389
	Internode	5.1597	4.8275	8.8212	3.9602	3.8431
K_2	Diameter	−1.2255	−0.0094	−0.1379	0.1194	−0.2205
	Internode	0.0526	0.6267	0.2213	4.3687	1.8820
Skewness	Diameter	−0.2639	−0.0629	−0.3831	−0.1598	−0.3032
	Internode	0.1265	0.3473	0.2678	0.3875	0.3463
Mode	Diameter	7.0920	7.6678 8.12%	9.0207 27.19%	10.5744 49.14%	11.1441 57.13%
	Internode	531.9895	669.6903 25.88%	706.4650 32.79%	1030.2662 93.66%	1310.3061146.30%
Standard deviation	Diameter	1.3128±0.0500	1.3336±0.0192	1.5163±0.0185	1.8256±0.0354	1.8239±0.0198
	Internode	139.1366±5.3071	157.3944±2.2665	161.6199±1.9804	229.9173±4.4604	351.2862±3.8276
Correlation r between	Diameter... Internode...	.0797±0.0535	0.1947±0.0195	0.1480±0.0169	0.3399±0.0242	0.3456±0.0136
Mean	Diameter	6.7475±0.0707	7.5843±0.0332	8.4403±0.0263	10.2826±0.0501	10.5913±0.0281
	Internode	532.1656±7.4897	670.2370±3.2052	706.8977±2.8007	1031.1571±6.3043	1311.5344±5.4131

TABLE 2

Length of internodes. Under each group the first column gives the frequencies when the observed values are reduced to 1000. The second column (F) the observed number of frequencies.

INTER-NODAL LENGTH μ	GROUP I	F.	GROUP II	F.	GROUP III	F.	GROUP IV	F.	GROUP V	F.
150	25.5	4								
250	0	0	1.8	2	0.7	1				
350	114.6	18	14.6	16	11.2	17			0.5	1
450	242.0	38	86.6	95	54.8	83	1.7	1	2.6	5
550	382.2	60	260.7	286	192.1	291	9.9	6	3.1	6
650	152.9	24	278.9	306	264.0	400	29.8	18	6.3	12
750	38.2	6	174.1	191	233.7	354	100.8	61	28.7	55
850	19.1	3	93.9	103	134.0	203	145.5	88	56.9	109
950	19.1	3	54.7	60	66.7	101	216.5	131	90.3	173
1050	6.4	1	19.1	21	29.7	45	175.2	106	104.4	200
1150			11.9	13	6.6	10	122.3	74	131.0	251
1250			2.7	3	2.6	4	67.8	41	101.2	194
1350			0	0	2.0	3	59.5	36	124.7	239
1450			0	0	0	0	31.4	19	81.9	157
1550			0	0	0.7	1	16.5	10	73.1	140
1650			0.9	1	0	0	13.2	8	59.0	113
1750					0	0	6.6	4	47.0	90
1850					0	0	1.7	1	30.3	58
1950					0.7	1	0	0	22.4	43
2050					0.7	1	1.7	1	12.0	23
2150									5.7	11
2250									7.8	15
2350									4.7	9
2450									0.5	1
2550									1.6	3
2650									3.1	6
2750									0	0
2850									1.0	2
		157		1097		1515		605		1916

TABLE 3

Diameter of internodes. Under each group the first column gives frequencies when the observed values are reduced to 1000. The second column (F) the observed number of frequencies.

DIAMETER μ	GROUP I	F.	GROUP II	F.	GROUP III	F.	GROUP IV	F.	GROUP V	F.
3	6.4	1			0.7	1	5.0	3	0.5	1
4	12.7	2	6.4	7	4.6	7	0.0	0	0.5	1
5	146.5	23	62.0	68	37.6	57	1.7	1	4.7	9
6	286.6	45	140.4	154	70.0	106	24.8	15	11.0	21
7	305.7	48	263.5	289	163.0	247	59.5	36	50.1	96
8	146.5	23	289.9	318	206.6	313	39.7	24	78.3	150
9	44.6	7	138.6	152	194.7	295	124.0	75	82.5	158
10	51.0	8	96.6	106	298.4	452	343.8	208	264.6	507
11			2.7	3	13.2	20	143.8	87	137.8	264
12					9.2	14	148.8	90	226.0	433
13					2.0	3	76.0	46	114.8	220
14							28.1	17	25.6	49
15							5.0	3	3.7	7
		157		1097		1515		605		1916

b. Frequency Distributions. Chart I

An examination of the polygons which represent the observed data shows clearly that they deviate from the normal symmetrical figures to a considerable extent. Even those curves which come nearer to the symmetrical figure have the maximum ordinates too high to be fitted with the equation of the normal curve

$$y = \frac{a}{\sigma\sqrt{2\pi}} e^{-\frac{x^2}{2\sigma^2}}$$

An actual determination of the various constants from the data according to Pearson's method of the moment proves the correctness of our supposition, for in every case the values of β_1 and skewness deviate widely from zero, while in the normal symmetrical curve, these constants just mentioned should equal zero or be very near to it, and in addition the value of β_2 is not equal to 3.

When we classify our curves according to the corresponding values of the constants, the frequency distributions of the diameters should be represented by Pearson's curve of type 1, except in group 4, in which it will be represented by the curve of type 4.

On the other hand, the frequency distributions of the length of the internode in groups 1, 2 and 3 will be represented by the curve of type 4, while the remaining two groups are represented by the curve of type 6.

I have not calculated the theoretical values of the curves, since the observed curves as they are answer our present purpose. In plotting these curves, we reduced the total frequency on the basis of one thousand in each case. This not only renders a comparison easier, but at the same time we can get a clear notion as to the mode of a gradual transformation from small to larger values where the total number of the internodes is approximately constant throughout life (see p. 28 for the validity of this assumption).

Thus we assume that there are one thousand internodes in the nerve of the thigh, and these numbers are distributed in different stages of the growing period in the manner shown in figs. 1 (diameter) and 2 (internode).

Let us first examine the curves for the internode. In group 1 (see p. 24) the curve is much nearer to the symmetrical figure and the range of the length of the internodes extends only from 150 to 1050 micra. We notice here that the theoretical maximum ordinate corresponds with the internodal length of 532 micra; that is it stands nearly at the middle of the abscissa.

In group 2 we notice several changes when compared with the curve for group 1.

1. The total range of the variates has been increased and extended more towards the higher values (250 to 1650 micra).

2. The position of the node has moved from 532 in group 1, to 670 micra in group 2, and thus it is now situated at about one-third of the distance of the total range from the lower end.

3. Finally the shape of the curve shows a still greater deviation from the symmetry.

All these changes when compared with group 1 indicate that as a consequence of the growth of the frog, the internodes have grown in length, and therefore the shorter internodes which are so frequent in group 1, have become less frequent in this group.

With some insignificant modifications, the same statements apply to all the other curves. Finally in group 5 we see a very wide extension of variates from 350 to 2650 micra, and at the same time the ranges found in group 1 can only be found in this case in the lower third of the total range. The position of the mode has now moved from 532 to 1310 micra, indicating that as a whole, the modal value of the internode has increased 146 per cent when compared with group 1. From being nearly symmetrical, the curve itself has become highly skew. The increase in range of the variates may also be shown from the successive values of the standard deviation which increase gradually in round numbers from 139 micra in group 1 to 351 micra in group 5.

Let us examine now the curves for the diameters. For this character the distributions of the frequencies are very irregular as compared with the curves which illustrate the distributions of frequencies of the internodes.

Nevertheless in all fundamental features, the two records agree very nicely; that is the range of variates is smaller in group 1 than in group 5. The position of the mode moves gradually from a less diameter to a greater diameter; from 7 to 11 micra. The position of the entire curve moves frrom left to right. The only distinct difference which can be found in the curves of the internode and diameter lies in the fact that the total amount of increment during these five successive stages is considerably less in the latter than in the former, and in fact the internodes show almost three times as much increase as the diameters (see p. 28) and consequently the changes in the form of the curves for diameter from group 1 to group 5 is less conspicuous than in the case of the internode.

c. Mean and Standard Deviation

As has already been pointed out by both Boycott and Takahashi, a determination of the proper average is very difficult on account of possible modifications due to the technique. The larger the animal, the longer is the internode, and vice versâ. In the former case, the longer internode is easily broken and as a consequence the shorter internodes are more often measured than the longer ones, while in the smaller frogs such accidents are less frequent, and the resulting mean will therefore be much nearer to the true value in this case than in the case of the larger frogs. We cannot however eliminate this error but it must be kept in mind when we come to draw the final conclusions. Our observations yield the following mean values for each group:

		GROUP I	%	GROUP II	% inc.	GROUP III	% inc.	GROUP IV	% inc	GROUP V	% inc.
Mean	Diameter....	6.7	0.0	7.6	12.43	8.4	25.15	10.3	52.43	10.6	57.01
	Internode ...	532.2	0.0	670.2	25.94	706.9	30.95	1031.2	93.76	1311.5	146.45

As we should expect, the mean values for both diameter and internode increase from group 1 to group 5. In order to see how much increase both diameter and internode have made during these five stages, the two measurements in group 1 were compared with those of the succeeding stages. As is shown in the same table, the percentage increase for the diameter is far below that for the internode. In fact the total increment in the diameter of group 5 over that of group 1 is only slightly more than half of the value of group 1, while in the case of the internode, it amounts to more than one and a half times the value of group 1. This means of course that the internode has grown three times more rapidly than the diameter during the same period. Whether or not this relation of growth is also true for the growth of the thigh itself, when its width and length are compared, is an interesting point to be determined. At least one fact is true as will be shown later, that the growth rates of the internode and of the entire nerve are approximately equal.

As to the constancy of the number of internodes on a nerve fiber, I may quote first the view of Boycott from his original paper. Boycott says (p. 377):

It appears from the figures that there is a small increase in the number of internodes during the growth in the length of the nerve. The increase is small, yet it is regularly progressive. It may be due to ordinary errors of experiment, while on the other hand it may represent an increase which actually takes place. There is one special circumstance which renders it suspicious. This is the fact that the longer an internode the less likely is it to remain unbroken and capable of being traced throughout its length: the longer internodes will in this way not be measured as often as they should, and hence the average internodal length will be smaller than it should be proportionately to the increase in the length of the internodes, This would account for the increase which is seen in the table, but whether it is the whole explanation it is impossible to say. Assuming that the figures are in the main correct, it must be concluded that there is a small (and somewhat doubtful) increase in the number of internodes, though the main part of the total increase in length is due to an increase in the length of individual internodes.

In the summary he says again (p. 380):

The number of internodes thus remains approximately constant at all ages. There is a small increase in the total observed number; there are however reasons for thinking that this is due to errors of method.

This question of constancy in the number of internodes in the nerve cannot easily be decided until we have a strong reason to believe that the average value obtained for the internode from the given nerve is nearly correct. There is still another theoretical objection to considering the observed values as the average of the whole population, when a small fraction of the lower end of the nerve only is examined (see p. 40). I have also tested this main point by using mean values obtained by the present biometric method, and the following are the results.

TABLE 4

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Sciatic length in mm.	16.25	21.07	25.60	34.10	48.17
Mean internodal length in μ	532.2	670.2	706.9	1031.2	1311.5
Number of internodes	30.53	31.43	36.21	33.07	36.73
Percentage increase		2.94	18.60	8.32	20.31

When the figures given above are considered, they show a gradual increase in the number of internodes as the nerves grow longer, the significance of which depends of course on the correctness of the mean values. This method of treatment is crude however, owing to the fact that the observed length of the internode for any given diameter differs more or less from the theoretical value. There are some reasons to believe that the theoretical are preferable to raw mean values for this determination. When we employ the theoretical values of the length of the internodes, the results are as follows:

TABLE 5

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Theoretical length of the internode	531.8	678.8	684.4	988.7	1360.6
Number of nodes	30.56	31.04	37.40	34.50	35.40
Percentage increase	0	1.57	22.38	12.89	15.83

From the foregoing we see that whether we use the raw mean length or the corrected mean length, the results are approximately the same; that is the data show an increase in the number of the internodes as the nerve becomes longer. In fact the total gain in the number of the internodes, when group 1 is compared with group 5, amounts to as much as 16 per cent. Even if we assume that the total number of measurements in group 1 was too small (157 observations) to trust its validity, the difference between group 2 (1097 observations) and group 5 amounts to as high as 14 per cent.

We can test this conclusion still another way; since the percentage increase in the internodal length when the mean length in group 1 is compared with that of group 5, is approximately 150 per cent, while the gain in the length of nerve runs as high as 200 per cent, thus the length of the nerve increases faster than that of the internode.

In this connection it is a matter of interest to note that the average number of the internodes is 33.8 in the present case, while Boycott has obtained 34.5. Although the present method of obtaining the mean values was entirely different from that used by Boycott, nevertheless the results show a difference of less than 1.5 per cent, indicating a close agreement between the simpler and more complicated treatment. Therefore we are forced to conclude as Boycott did, that so far as our present data are concerned, the number of the internodes increases with the advancing ages of the frogs.

It is however extremely important before drawing any final conclusions from these data to consider at least the two following points:

1. Technical difficulty in measuring the longer internodes as often as the shorter. This has been already discussed.
2. Number of newly added fibers.

This is certainly an important point to be considered.

Takahashi commented on this point thus:

> It follows from the foregoing result that so long as the nerve receives new (young) fibers, there will always be internodes which are relatively short, since they belong to fibers which have been subjected to the lengthening process for only a short time. The presence of these fibers reduces the average length of the internodes, and hence accounts in part at least for Boycott's observation that on the average the lengthening of the internodes in the sciatic nerve is slightly less than that of the nerve itself.

Both these points require further study.

d. On the Correlation in Growth Between the Internodal Length and Diameter

It has been stated by the previous investigators that the length of the internode varies with the diameter of the fiber in the sense that the fibers of greater diameter have the longer internodes. According to Birge ('82) during growth the average diameter of the fibers in the frog increases, and the average internodal length also becomes greater. In general, the results just mentioned are in harmony with the findings of Boycott and Takahashi. In this connection however, Boycott has drawn attention to an important point. He stated that

> an examination of the table will show however that *the internodal length increases proportionately more than the diameter,* so that in a large frog, the length of the internodes in the fiber of given diameter, is greater than in a small frog (p. 372).

Later Takahashi advanced this conclusion of Boycott a step further stating that

> in the same frog, *the length of the internodes* at different levels on fiber of like diameter in the nerves to the leg, *increases towards the periphery* This increase appears to be associated with the more rapid growth of the distal segments of the leg, but the influence of the segment on the portion of the nerve within it, is less marked as the frogs become larger.

Thus Boycott's and Takahashi's investigations reveal that the relation between diameter and internodes is far more complicated than it was considered to be by the earlier investigators, and varies not only when the different sized frogs are compared, but also at the different levels in the leg of the same frog.

I shall discuss this problem under two heads:

1. The correlation of the length and diameter of the internode from a single locality in a given frog.

2. The relation between the length of the internode and its diameter.

a. From a single locality in frogs of different sizes.

b. In the different segments of the leg of the same frog.

1. Correlation of the Length and Diameter of the Internode from a Single Locality in a Given Frog

In order to see whether or not the length of the internode is correlated with the diameter, I have determined the coefficient of correlation between the two characters just mentioned in each group from the formula given below

$$r = \left(\frac{\Sigma\, x'\, y'}{n} - v'\, v'' \right) \frac{1}{\sigma_1\, \sigma_2}$$

(see Davenport's Statistical Methods, 1904) and obtained the following results:

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Coefficient of correlation or r =	.0797 ± .0535	0.1947 ± .0195	0.1480 ± .0169	.3399 ± .0242	.3456 ± .0136

In every case the coefficient of correlation is positive and is greater than the corresponding probable error. We therefore consider that the diameter and the length of the internode are certainly correlated. Though the degree of correlation is never high, nevertheless we can say positively that so far as the present data are concerned, the longer internode is associated with larger diameter in the values given in each group, and vice versâ.

We also noticed in the above table that in general the degree of correlation becomes greater as the animal becomes larger. In another trial we have obtained from Takahasi's data (measurements from sciatic in thigh only) a coefficient of correlation as high as 0.6681, his observations having been made on large frogs (body weight 26 to 63 grams). Since during the period of rapid growth the relation between the two characters must be more irregular, it follows that the degree of correlation would be less in the young than in the adult. Therefore the view maintained by the earlier investigators that "the length of the internodes

varies with the diameter of the fiber in the sense that the fibers of greater diameter have the longer internodes" is correct when the fibers are from the same segment of the same animal.

This conclusion is still true even when all the measurements in the five groups are added together, since in this case again the greater diameter is still associated with the longer internode, though the degree of correlation is lessened.

The average of the coefficients of correlation in the five groups is found to be 0.2216.

2. The Relation Between the Internodal Length and Diameter

a. From a Single Locality in Frogs of Different Sizes

We have shown already that the diameter varies with the internodal length in the same animal (this is true either for an individual or for averages of several individuals) though the degree of correlation is not high.

When however, several frogs of different sizes are compared with each other, the internodal length varies, as has been found by Boycott, proportionately more than the diameter, so that in a large frog the length of the internodes on a fiber of given diameter is greater than in a small frog. This finding of Boycott can plainly be seen when one examines either the tables or charts of his original paper. I have also prepared by a different method the tables and charts to demonstrate this point.

The following table shows the observed values of the internodal lengths corresponding to the different values of diameters in all of the five groups of Boycott. These values have been determined from the correlation tables (see tables 10-14, pp. 45-47) the internodal length being the means of arrays corresponding to the various values of the diameter.

TABLE 6

DIAMETER μ	GROUP 1 μ	GROUP 2 μ	GROUP 3 μ	GROUP 4 μ	GROUP 5 μ
3	450	—	350	517	550
4	150	579	536	—	950
5	515	571	561	750	728
6	521	608	606	870	1055
7	558	683	655	900	1039
8	541	698	724	892	1123
9	464	705	718	941	1200
10	625	660	759	1025	1306
11		817	750	1090	1373
12			757	1129	1384
13			617	1113	1420
14				1068	1523
15				1050	1993

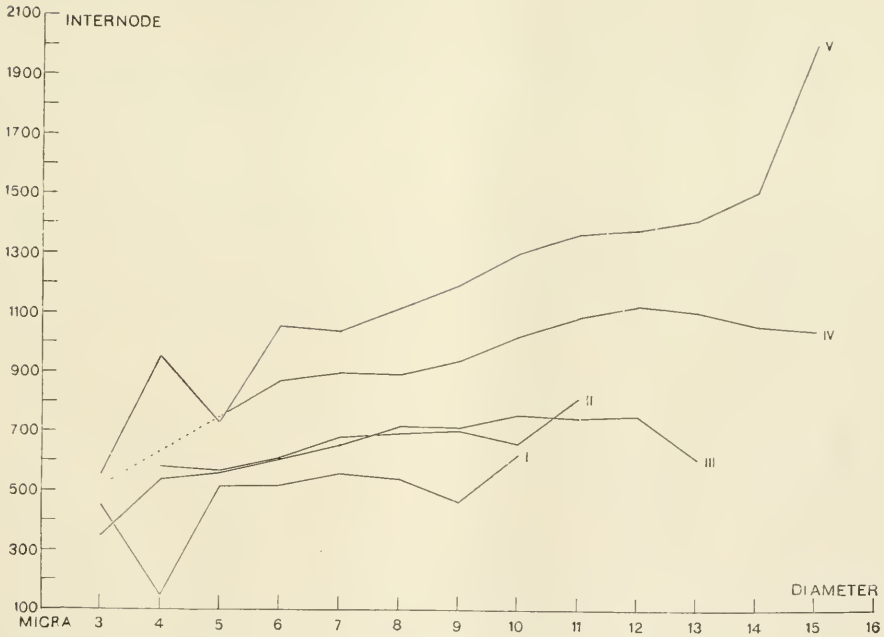

CHART 2. Showing the observed values of the internodal lengths corresponding to the different values of the diameters in all of the five groups of Boycott.

Let us first examine the curves (see chart 2). Although the curves are irregular at the ends where the number of observations is not large, yet each can be seen to follow a characteristic course when the middle portion is examined. We notice, as Boycott found, that the length of the internode varies proportionately more than the diameter, since the length of the internode becomes longer for a given diameter as the frogs become larger. In addition these curves are not parallel to one another but diverge more from the curve of group 1 as the frog becomes larger. This divergence in the course of the curves would of course mean that the relative amount of the increment to the internode following to the increase in diameter is not equal, but is greater for the larger frog than for the smaller frog. Since the smaller frog has a shorter internode for a given diameter than in the larger frog, it follows that the longer internode gains in increment proportionately more than the shorter internode. This relation suggests that the rate of increment in the length of the internode may be proportional to the length of the internode itself.

In mathematical terms this corresponds to the expression

$$\frac{dy}{dx} = hy$$

where x and y represent the diameter and the length of the internode respectively, and h is a constant.

In order to test this hypothesis the equation has been solved in the following manner:—

$$\frac{dy}{dx} = hy, \qquad \frac{dy}{y} = h\,dx$$

The integration of both terms gives at once the required formula.

$$\int \frac{dy}{y} = h\int dx + k \quad \text{or } \log y = hx + k \quad \text{or finally } y = A\,e^{hx} \quad (1)$$

$$\text{where } A = e^{k}.$$

Therefore if our hypothesis is correct, the foregoing exponential equation should adequately express the relation between the

length of the internode and its diameter in frogs of any given size.

Assuming that our hypothesis is correct, the next step was the actual determination of the two constants h and A from the observations for each group. I have determined these constants by ordinary algebraic methods, and the five equations stand as follows:

TABLE 7

For group 1 ..... $y = 477.6\ e^{.0159x}$
For group 2 ..... $y = 501.1\ e^{.0400x}$
For group 3 ..... $y = 470.2\ e^{.0426x}$
For group 4 ..... $y = 559.9\ e^{.0553x}$
For group 5 ..... $y = 613.5\ e^{.0752x}$

The following table (8) shows the values of the observed and calculated length of the internodes corresponding to the various values of the diameter. The calculations have been made for each group by the formulas just given.

TABLE 8

DIAMETER μ	GROUP I		GROUP II		GROUP III		GROUP IV		GROUP V	
	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated
3	450	501	—	—	350	534	517	660	550	769
4.....	150	509	579	565	536	558	—	699	950	829
5.....	515	518	571	588	561	582	750	738	728	894
6.....	521	525	608	612	606	607	870	780	1055	963
7.....	558	533	683	637	655	639	900	825	1039	1039
8	541	542	698	663	724	661	892	871	1123	1118
9.....	464	551	705	690	718	690	941	921	1200	1207
10.....	625	560	660	718	759	720	1025	973	1306	1301
11.....			817	747	750	751	1090	1029	1373	1403
12.....					757	784	1129	1087	1384	1513
13.....					617	818	1113	1149	1420	1631
14.....							1068	1215	1523	1758
15.....							1050	1283	1993	1896

In the first place if we examine the formula for each group, one point of interest is shown, that is the value of the constant "h" which determines the steepness of the curve (see formula 1) increases regularly from the smaller to the larger frog. Since,

when the value of this constant is equal to zero, the resulting curve will be parallel to the abscissa with the distance corresponding to the value of the constant A, therefore the increase in the constant "h" means that the curve becomes steeper as the frog becomes larger, at the same time with an increasing distance of the entire curve from the base line. For the latter statement we find one exception in group 3, in which the value of the constant "A" is smaller than that of the smallest frog. If however we examine the observed mean values in that group, a peculiarity can be found at once; that is the mean values in both the lower and upper ends of the curve are too small compared with the values found in the middle. Since our determination of the constants is based on the observations, such an aberrant result for this particular group is inevitable. Nevertheless, the higher value of the other constant "h" indicates a higher probability that all the mean values for group 3 should have been greater than they actually were.

Therefore with a single exception in the one constant in group 3, the equations are in harmony with the general feature in the growth of the internodes. When we come to an actual test, the observed values are too irregular to make valuable a detailed comparison with the corresponding values obtained by the equation.

On account of the difficulty just mentioned, the question of fit between the theoretical values and the observed can best be judged by an actual comparison of the two graphical representations of the values (see chart 3).

As we notice from the curves, the fit of the theoretical curve to the observed is very satisfactory in the first three groups, and the continuous lines run about the middle of the observed points. In groups 4 and 5 it is not as good as in the former three groups, nevertheless when the irregularity of the observed values in these two groups is considered, the continuous lines should be regarded as the best approximation to the observed values. At least the continuous lines run very close to the observation in the best part of the curve, that is the middle portion where the number of observations is large.

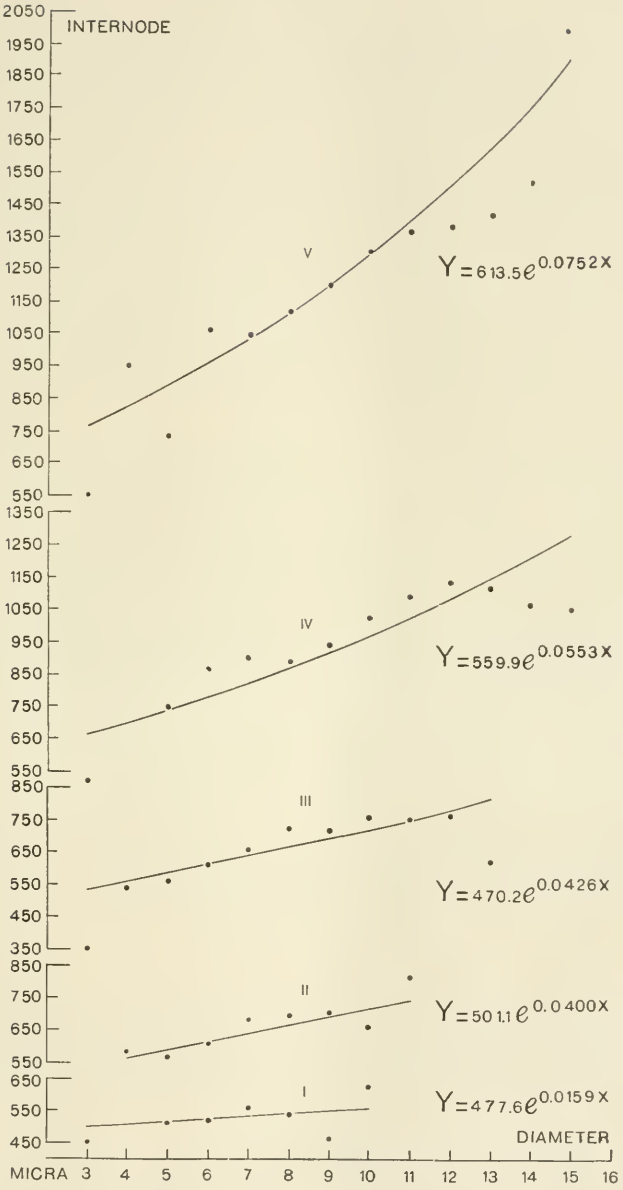

CHART 3. Showing the theoretical and observed values for the length of the internodes in all the five groups. The dots show the observed mean values, and the continuous lines the theoretical values as calculated by the formulas in table.

Obviously no simple curve will represent the system of points shown in group 4 and 5 better than the continuous lines obtained by the exponential equation. There is no doubt that the diminishing value of the ordinates following the increase in diameter, to be seen at the right end of the curves in groups 3 and 4, must be due to the small number of observations, as well as high irregularity in the length of the node, since it is not only opposed to the general results, but the three remaining curves show contrary relations. We are justified in assuming then that the relation between the length of the internode and diameter in different sized frogs is exponential, as it was supposed to be, and the law can now be stated as follows:

The rate of increment in the length of the internode following the increase in diameter is proportional to the length of the internode itself or

$$\frac{dy}{dx} = h\,y.$$

In this connection we have tested three more hypotheses touching the rate of increment in the length of the internode.

1. Namely that the rate of increment is proportional to the diameter:

$$\frac{dy}{dx} = hx$$

2. That the rate of increment is proportional to the product of the length of the internode and diameter:

$$\frac{dy}{dx} = h\,xy$$

3. That the rate of increment is proportional to the quotient of the length of the internode when divided by the diameter:

$$\frac{dy}{dx} = h\frac{y}{x}$$

When however these three equations were tested, it was found that so far as the constants are to be determined from the observations, all these equations give a very poor fit to the observed

data. Thus there is no doubt as to the justification of our first hypothesis that

$$\frac{dy}{dx} = hy$$

It follows from the foregoing analysis that Boycott's conclusion that "the internodal length increases proportionately more than the diameter" can now be put in the following way:

The length of the internode is proportional to the diameter in any growing stage of the frog in the sense that the former varies with the latter according to the relation given by the equation

$$y = A\, e^{hx} \qquad (1)$$

III. Takahashi's observations on the length of the internode in different segments of the leg of the same frog.

Takahashi found that in the same frog, the length of the internodes at different levels on fibers of like diameter in the nerves to the leg, increased towards the periphery. The following is the table quoted from Takahashi's paper (Takahashi '08, table 8):

TABLE 9

Showing the relative length of the internodes at T_3 compared with those at S_1, as a standard, in the case of the several diameter classes in all three frogs

	DIAMETER IN μ	LENGTH OF INTERNODES IN μ AT	
		S_1	T_3
Frog 3	4.0	425	578
	5.3	530	714
	6.3	578	805
Frog 5	5.3	623	834
	6.3	645	917
	7.3	805	1039
Frog 8	5.3	711	837
	6.3	828	963
	7.3	885	1001
Averages	5.9	659	855

The above table shows clearly that for any given diameter the length of the internodes which are nearer to the proximal end of the leg are shorter than those away from it. This observation suggests the possible existence of an exponential relation between the internodes from different segments of the leg in the same frog. In order to investigate this point, the total averages were taken as shown in the above table. I took this average for the reason that first, all frogs are mature, and second, on account of higher variability of the length of the internode, the sum of the larger number is important. From the average we see that the diameter which is approximately 6 micra gives 659 micra for the internode at the upper end of the thigh, and 855 micra for the internode found at the upper portion of the foot (cruro-tarsal joint), that is the relation between the two is 1:1.29.

To determine whether or not a similar relation can also be found between the length and diameter of the internode in different sized frogs, I selected two values of the internode for the diameter of 6 micra; one from group 4 and the other from group 5, as these two mean lengths are the nearest values giving a diameter comparable with the averaged figures in Takahashi's table. We have here 780 micra and 963 micra for the internodes in groups 4 and 5 respectively.[2] In this case the proportion between the two internodes is 1 : 1.23 as contrasted with 1 : 1.29 in the other.

These two ratios agree very well for the diameter of 6 micra. This agreement is necessary for the argument, but does not permit us to conclude that the relation between the diameter and the length of the internode in the different segments of the leg of the same frog is also exponential, since we cannot determine the form of the curve. For this reason we must seek for further evidence.

An examination of the figs. 3 to 6 in Takahashi's paper gives us an additional reason for the above conclusion, since there we find that not only the length of the internode for a given diameter increases towards the periphery, but the three lines repre-

[2] Takahashi has pointed out that the internodes on the fibers from the American frog, R. pipiens, are shorter than those found by Boycott on the fibers of R. temporaria (fusca).

senting the diameters of 5.3, 6.3 and 7.3 micra respectively are not parallel, but show a slight divergence (see also Takahashi '08, tables 4 to 8). The amount of divergence is very slight, but is regularly greater for the line corresponding to the greater diameter. This relation agrees with that found to exist between the two characters in the frogs of different sizes. We feel justified therefore in concluding that our hypothesis that the relation between the diameter and the length of the internode is exponential, even when applied to the length of the internodes from the different segments of the leg of the same frog, is correct.

On the basis of the preceding argument, we present the following final conclusion:—The exponential equation

$$y = A\ e^{hx}$$

expresses the relation between the length of the internode and its diameter either in different segments of the leg of the same frog, or in frogs of different sizes. This law seems applicable to both Rana pipiens and Rana temporaria (fusca).

Thus far we have merely demonstrated that when the data are examined, the relation existing between the two characters under consideration is adequately expressed by an exponential formula, but we do not make any inference as to the immediate factors which bring the two characters during the growth period into such exponential relation. As one of the factors, Takahashi counts the segmental influence, by which I understand the elongation of the internodes of Ranvier depend on the elongation of the segments of the limb in which they are found.

If this view of Takahashi is correct, we shall be justified in concluding that the growth of the segments in Rana pipiens follows also an exponential formula. Although unfortunately I have not sufficient data to test this point just mentioned in either Rana pipiens or Rana temporaria, I find it to be true at least in the case of the leg of the toad as shown by the recent investigation of Kellicott on Bufo lentiginosus (1907) in which the rate of increment in the length of the segments is proportional to the length of the segment itself.

This relation has been determined from the coefficients of correlation and standard deviation given in his paper. We assumed of course that the regression between the body length and length of thigh, shank and foot is linear. This is admittedly a crude method, nevertheless so far as we wish at the present moment to determine the general features of the curves, such simple treatment answers the purpose.

We have however one important difference between the segments of Rana and those of Bufo, in the fact that in Rana the length of the segment regularly increases towards the periphery, while in Bufo, the length of the shank is least and that of the thigh and of the foot stand in the order named. Thus in the toad if the growth of the internodes is not at all influenced by the elongation of the segments, we shall find a progressive increase in the length of the internodes towards the periphery, and the exponential law in this case will apply to the entire extent of the leg, while on the other hand if the segmental influence is the main determining factor, we shall find the shortest mean length of the internode in the shank, and the exponential law in this case will be applicable to each segment independently.

Therefore the question of general segmental influence as the main determining factor of the length of the internode in the different segments, can best be tested from the internodes in the nerve of the different segments in the leg of the toad. I hope to be able to take up this question in the near future.

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CORRELATION TABLES.

TABLE 10

Showing correlation of length and diameter of internodes in micra

GROUP I

Diameter / Internode	100–200μ	200–300	300–400	400–500	500–600	600–700	700–800	800–900	900–1000	1000–1100	Totals
3μ				1							1
4	2										2
5			3	7	9	3	1				23
6	1		7	12	15	8			1	1	45
7			4	8	23	8	3	2			48
8	1		3	3	10	3	2		1		23
9				6	1						7
10			1	1	2	2		1	1		8
Totals	4	0	18	38	60	24	6	3	3	1	157

TABLE 11

Showing correlation of length and diameter of internodes in micra

GROUP II

Diameter / Internode	200–300μ	300–400	400–500	500–600	600–700	700–800	800–900	900–1000	1000–1100	1100–1200	1200–1300	1300–1400	1400–1500	1500–1600	1600–1700	Totals
4μ		1	2	2			2									7
5		4	20	18	15	7	3	1								68
6		2	30	59	32	15	9	6							1	154
7		4	18	71	85	53	33	11	9	5						289
8		5	13	68	106	57	31	22	7	6	3					318
9	1		7	39	30	37	18	14	5	1						152
10	1		5	29	37	22	6	5		1						106
11					1		1	1								3
Totals	2	16	95	286	306	191	103	60	21	13	3	0	0	0	1	1097

TABLE 12

Showing correlation of length and diameter of internodes in micra

GROUP III

Diameter / Internode	200–300μ	300–400	400–500	500–600	600–700	700–800	800–900	900–1000	1000–1100	1100–1200	1200–1300	1300–1400	1400–1500	1500–1600	1600–1700	1700–1800	1800–1900	1900–2000	2000–2100	Totals.
3μ		1																		1
4		3			3	1														7
5		4	14	23	8	5	2	1												57
6	1	6	19	29	22	17	11	1												106
7			20	81	77	29	23	12	3	2										247
8		2	16	57	80	73	37	24	16	4	4									315
9		1	8	44	88	87	40	18	6	2				1						295
10			4	55	111	134	80	41	20	2		3						1	1	452
11			1	1	5	4	8	1												20
12				1	5	3	2	3												14
13			1		1	1														3
Totals	1	17	83	291	400	354	203	101	45	10	4	3	0	1	0	0	0	1	1	1515

TABLE 13

Showing correlation of length and diameter of internodes in micra

GROUP IV

Diameter / Internode.	400–500μ	500–600	600–700	700–800	800–900	900–1000	1000–1100	1100–1200	1200–1300	1300–1400	1400–1500	1500–1600	1600–1700	1700–1800	1800–1900	1900–2000	2000–2100	Totals
3μ	1	2																3
4																		0
5				1														1
6		1		4	3	4	3											15
7			6	5	7	7	6	4	1									36
8		2		7	5	2	4	3	1									24
9		1	4	6	17	24	13	7	1	2								75
10			5	22	33	50	33	21	14	17	6	2	2	2				208
11			1	10	12	10	15	14	10	3	5	3	3	1				87
12				4	6	17	19	14	10	11	3	4	1	1				90
13			2	2	4	11	6	8	2	3	4	1	2		1			46
14					1	5	6	2	2		1							17
15						1	1	1										3
Totals	1	6	18	61	88	131	106	74	41	36	19	10	8	4	1	0	1	605

TABLE 14

Showing correlation of length and diameter of internodes in micra

GROUP V

Diameter / Internode	300–400μ	400–500	500–600	600–700	700–800	800–900	900–1000	1000–1100	1100–1200	1200–1300	1300–1400	1400–1500	1500–1600	1600–1700	1700–1800	1800–1900	1900–2000	2000–2100	2100–2200	2200–2300	2300–2400	2400–2500	2500–2600	2600–2700	2700–2800	2800–2900	Totals
3μ			1																								1
4							1																				1
5		1	1	3	1	1	1	1																			9
6		1			1	5	3	4	2	1	1	2				1											21
7			1	3	11	16	20	12	11	7	4	3	6	2													96
8	1	3		3	9	16	20	25	24	14	12	7	7	2	3	1	1		1		1						150
9			1	1	10	12	17	25	25	13	19	11	4	8	5	4	2			1							158
10				2	15	32	46	40	72	56	69	46	41	37	18	11	9	3	2	3	1		1	3			507
11			2		5	12	15	21	26	34	42	19	24	19	14	10	9	6	4	2							264
12					2	6	28	58	70	44	44	40	33	28	30	16	14	6		6	4	1	1	1		1	433
13					1	7	16	12	20	23	45	23	18	12	13	8	5	8	3	3	2			1			220
14						2	6	2	1	2	3	6	7	4	4	6	3		1				1	1			49
15														1	3	1					1					1	7
Totals	1	5	6	12	55	109	173	200	251	194	239	157	140	113	90	58	43	23	11	15	9	1	3	6	0	2	1916

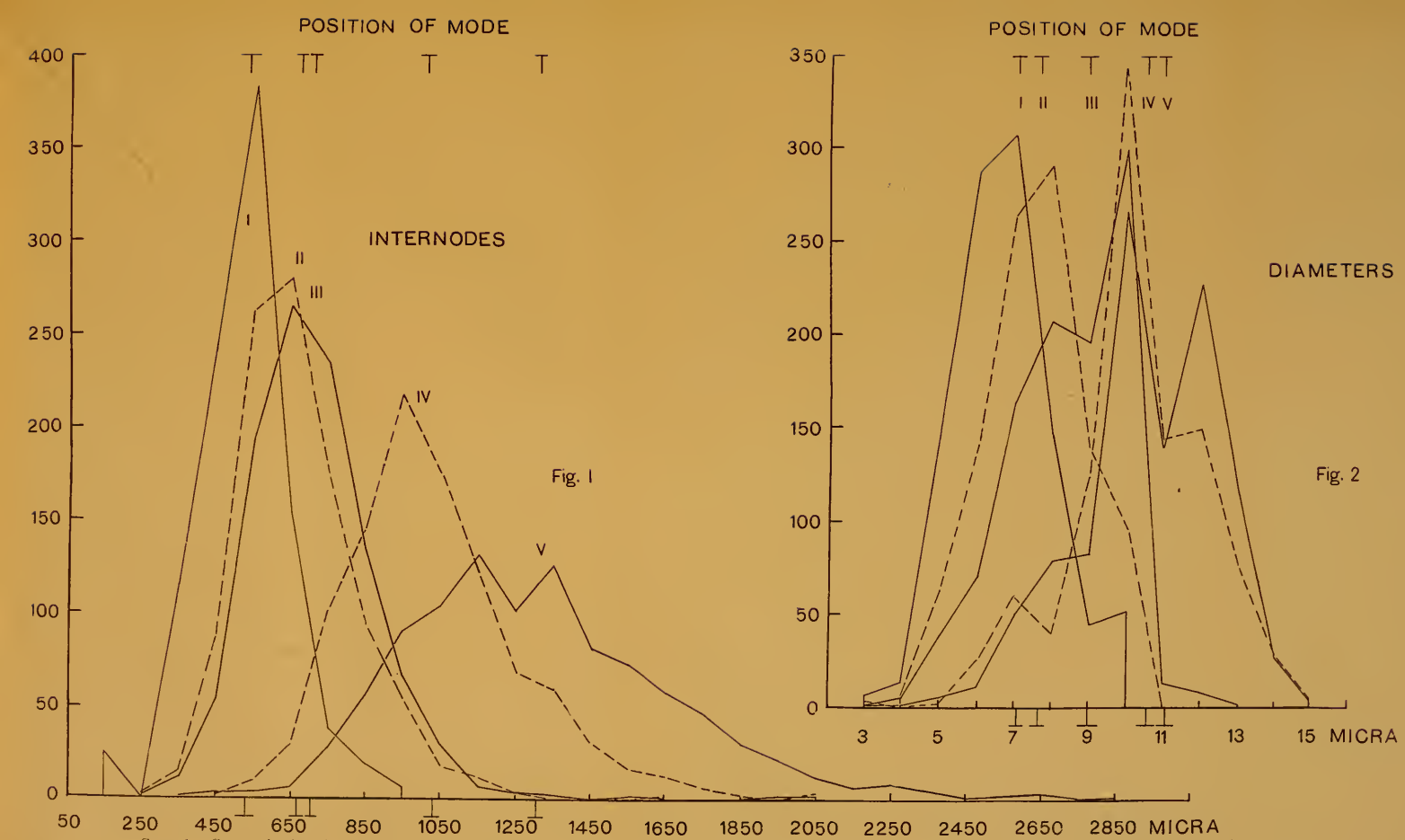

CHART 1. Curves showing the distribution of frequencies of the several lengths of the internodes and diameters for all of the five groups. Fig. 1, Internodes. Fig. 2. Diameters.

THE OLFACTORY NERVE, THE NERVUS TERMINALIS AND THE PRE-OPTIC SYMPATHETIC SYSTEM IN AMIA CALVA, L.

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From the Anatomical Laboratory of the University of Chicago

WITH THIRTY-FIVE FIGURES

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There are two main reasons why Amia was selected for a study of its nervous system. It is an archaic form likely to show interesting relationships with the sharks and other lower vertebrates. In the second place, it is relatively abundant in the region where the work was first undertaken, so that it could be procured in numbers to satisfy the demands of the neurological methods. It was our original intention to describe the anterior part of the central nervous system and thus supplement the work done by Kingsbury ('97), on the medulla oblongata and by Allis ('97) on the peripheral nerves of Amia, but when it was found that there are large numbers of ganglionic nerve cells among the olfactory fibers in the adult nasal capsules and that these cells are derived from the ganglion which Allis described in the young Amia, it was thought best to study first the peripheral olfactory apparatus. The work on the central nervous system has been deferred for a later paper. The present article includes a study of the em-

bryology and the adult morphology of the olfactory apparatus in Amia with some comparative work on Lepidosteus and a few teleosts. It has seemed best to include a description of certain sympathetic nerves and ganglia found in the meninges of the forebrain of Amia.

MATERIAL AND METHODS

The demands of the various kinds of neurological technique have required the use of a large number of young and adult Amia. At one time time 450 young Amia from 60 mm. to 75 mm. in length were employed for Golgi preparations. In addition to the neurological preparations of young and adult Amia, a great many embryos and young from two days before hatching to 100 mm. in length were sectioned, mounted serially, and stained for cytological results. Most of these sections were stained with Heidenhain's iron hæmatoxylin, but others were stained with Delafield's hæmatoxylin and by Weigert's method. Much of the first material which was available to me for cytological preparations of embryos had been fixed in bichromate-acetic fluid or in formalin. Later, when embryos and young were collected they were fixed in Zenker's fluid.

Some of the neurological methods which gave the best results in my hands will be indicated briefly. The rapid process of Golgi's method (Lee's Vade Mecum, sixth edition, p. 437) gave the best results. Double impregnation was often employed to good advantage. Good results were sometimes procured on adult brains by a preliminary fixation for from five to twelve hours in four per cent formaldehyde neutralized with lithium carbonate or with ammonia. The treatment with neutral formalin seemed to favor the impregnation of axones.

In order to cut serial sections of the Golgi preparations the pieces were imbedded in paraffin melting at about 50° C. Xylol, which dissolves the silver precipitate, was avoided and the clearing done in cedar oil. The cedar oil was used repeatedly and is probably all the better for being saturated with silver after use the first time. The cedar oil was warmed over the imbedding oven

after each use for clearing, in order to evaporate the alcohol introduced. Dehydration was accomplished by passing the pieces quickly through the lower grades of alcohol, which have a tendency to dissolve the silver precipitate, and was made complete by two changes of 95 per cent, and of alsolute alcohol at intervals of about two hours. Pieces were finally left in cedar oil for twelve to twenty-four hours. From the cedar oil the pieces were transferred to melted paraffin which was changed three times at intervals of three or four hours in order to have the blocks free of cedar oil. By lowering an electric light bulb over the block while cutting in cool weather, sections more than 50 micra thick can be cut in ribbons with ease.

Series of adult brains by the Weigert method were cut in three different planes and a transverse series of the head of a young Amia 100 mm. in total length was made. The iron hæmatoxylin method recommended by Houser ('01) gave better results than the Weigert method in the forebrain where there is little medullation. Bielschowsky's formalin-ammonia-silver method was tried with some success, but by far the best results were given by the Cajal ('05) treatment for the anterior part of the brain and for certain fibers in the olfactory capsules. For showing axones by the Cajal method, the best preparations were made after fixation in 95 per cent alcohol. In some cases a small amount of ammonia was added until there was a slight reaction to litmus paper. Material thus fixed may be kept in 80 per cent alcohol until it is convenient to employ the silver bath. Small pieces were kept warm in silver nitrate of about 2 per cent, for from three to five days. The same precautions were taken as for Golgi preparations to avoid xylol and the prolonged use of low grades of alcohol in embedding. Paton's ('07) modification of Bielschowsky's method was tried on embryos and young of Amia. It showed neurofibrils in none but the larger elements of the brain in the later stages after hatching.

Nissl preparations were made of the brains of adults to show regional differentiation and to demonstrate the characters of the cells of the nervus terminalis. Material fixed in Graf's chrom-oxalic mixture cited by Houser ('01) gave better results than

either formalin or alcohol fixation. Toluidin blue gives a more brilliant stain than methylen blue and is easier to control, as it does not diftentiate so rapidly in the alcohol. Neither does it seem to fade so rapidly afrer the sections are mounted in balsam. Methylen blue was used intra-vitam by injecting it through the ventral aorta. One half of one per cent solution in distilled water was kept in stock and mixed with ten times its bulk of normal saline solution at the time of using. Chemically pure sodium chlorid was used with Grübler's Bx brand of methylen blue. The method employed was, in the main, the one used by Wilson ('04). Pieces were cut out and examined under the compound microscope to determine when impregnation had taken place. The pieces were kept in the ice box while in the ammonium molybdate and in the alcohols. This gave better results on the peripheral than on the central nervous system.

I wish to thank the Ohio State Academy of Science for a substantial contribution from the McMillin fund toward defraying the expense for material. I am much indebted to Mr. Alex. Nielsen of Venice, O., for furnishing me adult fishes fresh from his nets and to Prof. Herbert Osborn for facilities for working and gathering material while at the Ohio State University Lake Laboratory on Lake Erie during three summers. I am also under many obligations to Prof. C. Judson Herrick for generous contributions of literature loaned or donated, as well as for his friendly advice and criticism while engaged in the work.

HISTORICAL SKETCH

In 1862 Max Schultze showed that the nucleus of origin of the olfactory neurones of the first order lies peripherally in the olfactory epithelium. Since that time his results have been confirmed by various workers repeatedly. Bedford ('04) gives a brief summary of some of the more important papers,—especially those of an embryological nature. His résumé of the literature shows that the older embryologists were of the opinion that the olfactory nerve arises centrally. Then views changed after the appearance of the paper of His, Jr., in 1889, showing that in

the human embryo the olfactory nerve arises exclusively from the periphery. The view prevails at present that the olfactory nerve differs from all other nerves in vertebrates in that its cells arise and remain in the ectoderm. Bedford's investigations on embryo swine support this view.

It would seem from the brief account just given that the embryology of the olfactory nerve is simple, but Milnes Marshall, Balfour, von Kölliker, Beard, Chiarugi, Disse, and others have described a ganglion in the developing olfactory nerve. Some thought this ganglion came from the brain or from the forward continuation of the neural crest into the region of the olfactory nerve. His ('89) described the ganglion as coming from the periphery and contributing neuroblasts to the formation of the olfactory nerve. Disse ('96) says that the cells of the ganglion give rise to the sheath cells of the olfactory nerve. Bedford did not determine their fate in swine.

In 1895 Pinkus described a new nerve in Protopterus, which is intimately associated with the olfactory nerve. Soon afterward Allis ('97) found a nerve in Amia calva near the olfactory nerve. He noted its ganglion in the young and homologized the nerve with Pinkus' nerve in Protopterus. About two years later Locy ('99) found a nerve in sharks related peripherally with the olfactory mucosa. As it had a more dorsal connection with the brain than that described by Pinkus for Protopterus, he was not inclined to homologize the nerve in selachians with the one in Protopterus. He extended his observations to other sharks and found in some species a more ventral connection of the nerve with the brain. He then thought the nerve homologous in Protopterus, Amia and selachians ('03). When the nerve was found to be present in a large number of sharks Locy ('05) named it the *nervus terminalis*. He also treated this nerve as a part of the olfactory nerve when he first described its development in Squalus acanthias ('99), but later ('05) he came to consider it as an independent nerve.

Bing and Burckhardt ('04) described the nervus terminalis in adult Ceratodus where Sewertzoff ('02) had previously seen it in embryos. In all probability it has not been sufficiently sought

for in the remaining living lung-fish, Lepidosiren. Burckhardt, as cited by Pinkus ('05), found the nerve in Callorhynchus. Burckhardt has suggested that its ganglion might be homologous with the ganglion found by Rubaschkin ('02) in the chick, but Rubaschkin considered the ganglion in the chick to be developed as a part of the trigeminus nerve, in all probability.

Ernst DeVries ('05) described a ganglion in the course of the nerve fibers to the *organan vomeronasale* (Jacobson's organ) of human embryos of about three and a half to four months. In the guinea pig he found a similar embryonic ganglion. He expressed the opinion that the same relations probably exist throughout the whole series of vertebrates. Although the organon vomeronasale does not exist as such among a large number of anamniotes, he thinks the nerve described by Locy is probably homologous with the nerve to Jacobson's organ.

If the nervus terminalis of lower vertebrates is homologous with the nerve to Jacobson's organ or with some part of the nerve to this organ, we may well look for some evidence of a similar nerve or ganglion everywhere in the vertebrate series, for Jacobson's organ has been described as occuring embryologically or in the adult in forms from Amphibia to man. The literature shows that the nervus terminalis is almost universal among the living generalized fishes. We may have overlooked its presence in the remainder of the fishes. That such may be the case is indicated by the fact that the writer ('08) has found the ganglion among the fibers of the olfactory nerve of the young of two species of Lepidosteus in the identical relations described by Allis for Amia. No mention of such a ganglion has been found in the literature. Also, while this work was in progress the writer found ganglion cells indicating a nervus terminalis in the olfactory nerve of the carp, and Sheldon ('09) found its central connection with the brain. Sheldon and Brookover ('09) reported its presence in the carp at the Baltimore meeting of the American Association of Anatomists. Meanwhile, Herrick ('09) found the nervus terminalis in the tadpole and in the adult frog.

Pinkus ('05) in his third paper dealing with the nerve as found in fishes, summarizes its relations with the olfactory nerve by say-

ing there are three points of correspondence: (*a*) same structure, (*b*) same peripheral ending in the nasal mucous membrane, and (*c*) same termination in the prosencephalon. He thinks we cannot say anything definite as to its function at present. Johnston ('06, p. 106) suggests that it is probably a general cutaneous nerve. Allis ('97) noted that the ganglion in Amia develops at the same time as the ciliary ganglion and suggested that, since its cells resemble those of the sympathetic rather than cerebrospinal ganglia, the nerve is probably a sympathetic nerve.

Allis ('97) described each olfactory nerve of Amia as being made up of three bundles, of which the smaller, ventro-median, constitutes the nerve "*n*" of Pinkus. He states that there is an interchange of fibers between all three of the bundles in the young as well as in the adult. He says,

> In the adult the ventro-median bundle of the olfactorius is distributed, so far as macroscopic observations can show, to the nasal epithelium at the extreme anterior end of the nose. In embryos such is also its apparent distribution, though I have never been able in my preparations to trace it definitely to that tissue. During the larger part of its course, in 30 mm. to 50 mm. specimens, it is easily distinguished from the lateral bundles by the presence of the large round cells which Pinkus describes in Protopterus. Near the anterior end of the olfactorius, however, these cells disappear, and the fibers into which the bundle breaks up cannot be distinguished from the other terminal branches of the main nerve.

He continues by saying that centrally,

> The fibers of this bundle enter, in large part, with the fibers of the lateral bundles into the anterior end of the lobus.

A few of its fibers were traced by him in one series of sections caudally ventral of the brain membranes to enter the brain lateral to the recessus interolfactorius, which latter term he explains by saying,

> in Amia, in front of the lamina terminalis, or sulcus olfactorius, and hence into the lobus olfactorius and not into the forebrain proper (p. 512).

This latter statement of Allis is based on findings in sections of a single young specimen of Amia. He found this posterior bundle in gross dissections of the adult, but says he never could find how it ended in the brain. His illustrations (plate xxxvii) of the adult brain shows the posterior root of the nerve running well back between the optic chiasm and the brain. As I understand the terms "lobus" and "lobus olfactorius" employed by Allis in the above quotations from him, he uses them to indicate what neurologists now call the olfactory bulbs in which are located the mitral cells and their glomeruli. Olfactory lobes are now commonly used to indicate olfactory centers in the forebrain proper. Consequently, it would appear that Allis traced some of the fibers of the nervus terminalis in young Amia into the anterior end of the olfactory bulbs and others into the posterior ventral portions of the olfactory bulbs. He found the main bundle in the adult in the same position as in the young, but traced some of its fibers farther caudad without determining their ending. Locy ('03) examined the nerve in adult Amia and says it does not have the conspicuous separateness which characterizes it in selachians.

After examining a large number of adult Amia brains macroscopically I could never be absolutely certain that I saw anything different from olfactory fibers, which often break up into various small bundles before joining the olfactory bulbs. Anything that seemed to resemble the alleged posterior connection near the optic nerve, could never be distinguished with certainty, when cleared in xylol, from connective tissue or blood vessels. Kappers ('07) had no better results from the study of Weigert and Bielschowsky preparations of the brains of adult Amia which, however, had been removed from the cranial cavities before they came into his hands.

By examining the young stages of Amia in which Allis described the large cells that distinguished the nervus terminalis from the remaining olfactory bundles, it was easy for me to confirm his findings in the main. This led me to undertake a detailed examination of the early embryology of the nervus terminalis. As it had been described as a separate nerve by Locy ('05) in selachians, it occurred to me that it might be connected in Amia and Lepidos-

teus with the sucking disk or adhesive gland on the snout of the young which has been described by Dean ('97) and others; but on examination it was found that the sucking disk had already atrophied to a large extent before the ganglion can be recognized in the young.

In the description that follows, I shall employ the term nervus terminalis which has been largely used in the literature recently, although it will appear that I do not have any evidence that the nerve is separate from the olfactory nerve in the fishes which I have examined, and consequently I am led to the view that it is a part or component of the olfactory nerve, as Locy ('99) in his first work on its development in the sharks considered it.

EMBRYOLOGICAL DEVELOPMENT OF THE OLFACTORY ORGAN IN AMIA

The sections on which my study of the early embryology of the nasal pit is based were cut transversely or horizontally six micra thick and stained with iron hæmatoxylin and acid fuchsin. The series of embryos first described were all taken from a single nest from May 17 to May 20, 1908, and fixed in Zenker's fluid at intervals of three hours. Only such stages will be described as show differences worthy of note as compared with earlier stages.

At a period forty hours before hatching and consequently about eighty hours after fertilization, since it takes Amia eggs about five days to hatch, the nasal placodes are quite readily recognizable as solid masses of cells occupying much of the space between the optic cups posteriorly and the sucking disk anteriorly and ventrally. They are somewhat pearshaped with the smaller end pointing forward and downward between the two developing halves of the sucking disk with which they come into contact. At this stage the olfactory cups meet each other at their anterior ends in a common mass of cells (fig. 1) situated ventrally between the two developing halves of the sucking disk. The plane of the section figured slants upward and backward. There is between this unpaired portion of the olfactory placode, which is not far

from what will be the anterior portion of the roof of the mouth, and the neural tube, a small button of cells (fig. 1). No limiting membrane was made out between this group of cells and the brain wall. The cells were like those of what may be termed the unpaired olfactory placode into which they grade.

In a stage six hours older which has not been figured, the button appears as a cord of cells tapering downward and forward

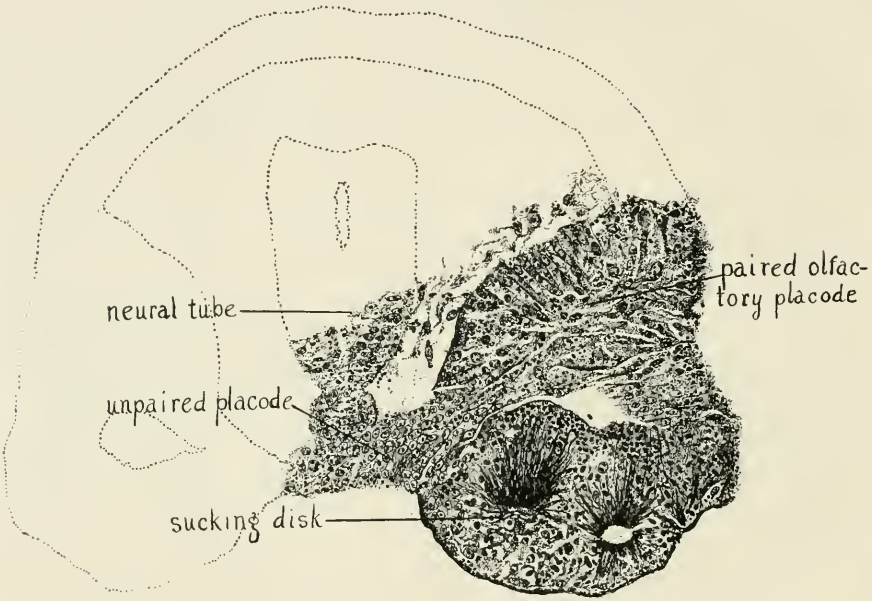

Fig. 1. Transverse section (slanting slightly upward and backward) of the anterior end of an embryo of Amia forty hours before hatching. Shows the paired and unpaired nasal placodes, the anterior end of the neural tube, and the sucking disk. Yolk granules are still evident in sucking disk. Iron hæmatoxylin and acid fuchsin, 6 micra thick. × 215.

from beneath the anterior end of the neural tube to the unpaired portion of the olfactory placode. A similar unpaired portion of the olfactory placode has been described by von Kupffer ('93) in the sturgeon. From this fact and the unpaired condition in Amphioxus and the Cyclostomes he argues for primitive monorhinism in the vertebrates and secondary amphirhinism. The

cord of cells just described in Amia would seem to correspond in position to the place where von Kupffer's *lobus olfactorius impar* ('93, fig. 15) comes into contact with his *mediane Riechplatte*. It was at first suggested to my mind that in Amia the cord of cells might be the ectodermal portion of the hypophysis, but a mass of cells believed to be Rathke's pouch is already present near the optic chiasm. Johnston ('05, p. 202) thinks the lobus olfactorius impar marks the dorsal border of the neuropore. As the first evidences of the nervus terminalis appear much later, no stages of Amia were cut at a date early enough to show the neuropore. Just as this goes to press Johnston ('09) publishes an article describing nerual crest near the olfactory placodes in lower vertebrates. The early development of the unpaired olfactory placode and of the neuropore should be investigated fully in Amia.

At about the middle of their antero-posterior extent the olfactory pits come into contact with the brain wall forty hours before hatching, when there is still an unpaired placode. This point of contact is some eight sections posterior to the anterior end of the neural tube, that is to say, about fifty micra. The connection is very slender, as it is comprised within two sections. It cannot be definitely stated that any fibers extend across from the placode to the brain at this time, but there is solution of the limiting membrane of the neural tube at this point. There is a delicate limiting membrane, shown by the fuchsin stain producing a red line, between the mesodermal elements and the caudodorsal part of the olfactory placodes, but none could be made out anteriorly and ventrally. The lack of a limiting membrane about the anterior ventral part of the olfactory placodes in the early stages was thought to be due to the recent connection of these placodes with the unpaired placode (fig. 1), and that no membrane has as yet formed in this position. Perhaps this may be the reason why olfactory fibers were observed to appear first at the anterior end of the placode, in most cases, when the olfactory nerve develops.

The condition of the olfactory placodes at a time six hours later than the stage described above, does not differ greatly and has

not been figured. At this time, which is thirty-four hours before hatching each of the paired placodes is still connected with the ectoderm at a point between the sucking disks, although by a more slender cord of cells than was the case in the earlier stage described. But the points where these two cords of cells reach the ectoderm adjacent to and between the two halves of the sucking disk, are now removed farther laterally from each other. From a position midway between the two halves of these two paired placodal connections with the roof of the mouth, there is still a strand of cells extending from the ectoderm to the anterior ventral edge of the tip of the neural tube. This cord of cells is largest near the neural tube. There is no appreciable increase in the size of the connection of the paired placodes with the brain over the previous stage described. A slight indentation of the cuticular ectoderm shows where the external opening of the nasal sac will appear later. The embryos showed slight motions at this age.

About twelve hours later than the last stage, or some twenty-two hours before hatching, there is no unpaired nasal placode nor any evidence of a cord of cells from the roof of the mouth to the brain wall at its anterior end. The paired placodes in one or two preparations of this stage have shown a slight elongation into a point forward just beneath the ectoderm. The nasal placodes, as well as the anterior end of the neural tube, are farther removed from the anterior end of the snout than was the case earlier. At this time there appears to be a fibrous connection between the olfactory capsules and the brain (fig. 2). Whether this connection is protoplasmic or is composed of true neurofibrils such as those described by Paton ('07), was not determined, as the method of Bielschowsky which was tried, did not differentiate any fibers in young or embryonic Amia. The connection is quite slender, as it is seen in only two sections, and is in consequence not more than twelve micra in thickness. A very few cells of the placode near the place of origin of the fibers are slightly larger than the others at this age. The distance from the placode to the outer brain wall is almost nothing at this age and there are no nuclei in the course of the olfactory nerve.

There is a definite membrane about the nasal capsule except where the olfactory nerve originates and for a slight distance anterior and ventral therefrom.

At a period three hours later, or about nineteen hours before hatching, a slight increase has taken place in the number of large cells in the placode near the origin of the olfactory fibers. The latter are not numerous, being confined to three or four sections

FIG. 2 Transverse section of a embryo Amia 22 hours before hatching. Shows contact of the paired olfactory placode with the neural tube. Preparation as above. × 450.

six micra thick. Some cells which have the characters of neuroblasts are now seen within the brain wall near the point where the brain has raised slightly to meet the olfactory nerve. This is the beginning of the olfactory bulbs. There are mitoses in the layer of germinative cells next the brain ventricle. Also, mitotic figures are seen here and there throughout the nasal placodes which have no lumen as yet. A slightly greater space exists at

this stage, than formerly, between the placode and the outer wall of the brain. Mesenchyme accompanied by capillaries and blood corpuscles has pushed into this space. An outer zone of nerve fibers has appeared just inside the brain wall and extends from a point anterior of the olfactory nerve to some distance posterior of it.

Three hours later there are many more fibers in the olfactory nerve but no nuclei in its course. In one preparation of this stage a very small cavity can be made out near the center of the placode. This cavity is not connected with the outside, but lies a little nearer the lateral side of the placode opposite the slight depression already mentioned as occurring in the epidermal layer of the ectoderm. The cells of the mesial side of the placode have assumed their columnar epithelial shape with nuclei at some distance from the lumen, as in the adult; but those on the opposite side of the lumen, where the external opening will appear later, are more rounded and irregular in arrangement. From this stage onward it is readily seen that the nasal capsule is continuous at its border just under the epidermis with the inner layer of the ectoderm in which taste-buds arise a little later. It can be said then, that at this age, some sixteen hours before hatching, the cells on the median side of the placode have assumed a more or less definite character of neuro-epithelium and it is probable that the olfactory nerve is composed of definite neurofibrils. The fuchsin stain shows a plainly fibrillar structure.

Other stages are about the same until six or seven hours before hatching, when in horizontal sections two bundles of fibers can be seen converging to a union just before entering the brain wall (fig. 3). The anterior one is slightly larger and is probably the older, as the earliest fibers are generally seen near the anterior border of the placode. Some three or four cells are seen among the olfactory fibers just as they leave the nasal capsule. Mesenchyme cells having capillaries and blood corpuscles in their midst, crowd near the nerve but in many of the preparations can be distinguished by their staining reactions from the cells inside the nerve. The two branches of the nerve can be followed well in among the nuclei of the nasal capsule. There is no external

opening to the capsule (fig. 3), although the central cavity is larger than when it appeared ten hours earlier.

At a period about six hours after hatching the olfactory nerve has lengthened to one fourth the diameter of the nasal capsule (fig. 4). A small increase is to be noted in the number of nuclei among the fibers as they arise from the capsule. The two main peripheral divisions of the olfactory nerve are more evident than previously. An outer fibrous membrane covers the brain

Fig. 3. Horizontal section of Amia 6 hours before hatching, showing anterior end of the neural tube, the olfactory placode with beginning of nasal cavity, and the origin of the olfactory nerve from the placode. Iron hæmatoxylin and eosin. × 450.

except where the olfactory fibers pierce it. A marginal zone of fibers lies just inside the brain at this point, almost entirely devoid of nuclei so that it does not seem possible that any nuclei are migrating from the neural tube into the nerve.

The olfactory capsules remain closed in this series of embryos until about thirty hours after hatching, although slight cavities are to be detected twenty-four hours earlier among the laterally

placed cells of the capsule. At this time the olfactory nerve has a large number of fibers and its length is equal to about half the diameter of the olfactory cup (fig. 5). The fibers spread peripherally, ramifying as small bundles among the cells of the capsule. The thickness of the capsular epithelium is not so great in the middle of the mesal side of the capsule. This is opposite

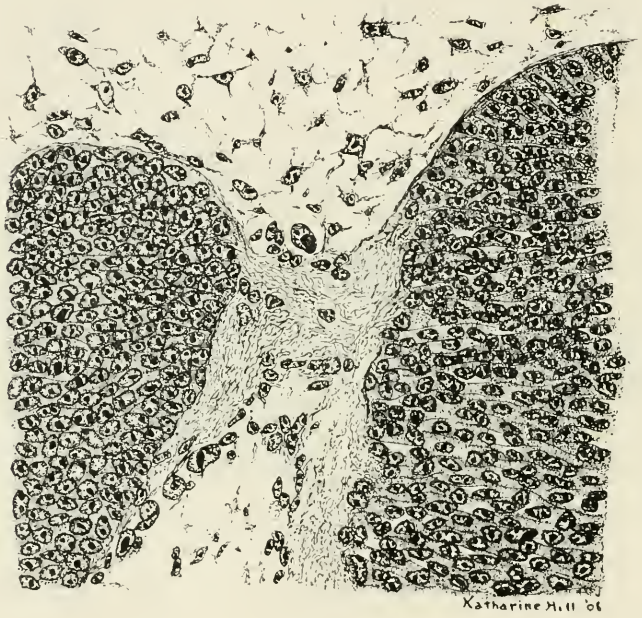

Fig. 4. Horizontal section of Amia 6 hours after hatching. Shows two rami of the olfactory nerve as it arises from the placode, the migrating placodal nuclei and the fiber zone devoid of neuroblasts just inside the neural tube. Stain as above. × 400.

the middle of the spread of the fibers into the nasal capsule, and causes the capsule to appear flattened laterally. The thin nasal epithelium at this point has more nearly its adult character than the epithelium at the margins of the capsule reached by the laterally spreading olfactory fibers (fig. 5). Laterally where the walls of the nasal capsule are thicker there are nuclei arranged

irregularly at the outer margin of the capsule. These irregularly arranged nuclei form a continuous series with the nuclei within the olfactory nerve, which have migrated out of the placode. At this time these nuclei appear to be nuclei of indifferent cells that are migrating along the olfactory nerve. Probably they are of the same general nature as the nuclei producing the sustentacular cells of the nasal epithelium. Later the larger part of these nuclei along the olfactory nerve produce sheath cells for

FIG. 5. Horizontal section of Amia about 30 hours after hatching. Shows growth in length of the olfactory nerve. Nuclei are migrating from the placode along the olfactory nerve. Note the position of the blood vessel at the anterior side of the olfactory nerve. Stained in thionin and acid fuchsin. × 400.

the olfactory fibers. They are more numerous peripherally than in previous stages, but disappear entirely just before the brain wall is reached. Inside the brain there is a fiber zone, as was the case earlier, that is devoid of nuclei (fig. 5). The mesodermal elements are closely crowded about the outside of the connective tissue sheath which is seen at the outer border of the nerve peripherally where it is continuous with the sheath around the nasal capsule. Where this sheath is present the mesodermal

elements are outside it and the nuclei within it among the nerve fibers belong to the sheath cells. This membrane, however, cannot always be made out. When this is the case, it is difficult to differentiate the mesenchyme cells from the cells among the fibers of the nerve. Their nuclei are about the same in size, but in favorable preparations stained with iron hæmatoxylin the nuclei of the mesodermal cells appear darker with a greater number of chrolmatin granules in them. The sheath cells within the nerve have a rather clear nucleus with one or two nucleoli.

Unfortunately an accident seems to have befallen the male fish guarding the nest from which the stages above described were taken and a turtle was found in possession of the nest about thirty hours after hatching. This necessitated taking eggs from a different nest for the later stages. This second series began during the second day after hatching and was continued for more than a week. They were preserved in Zenker's fluid at intervals of about four hours for the first two days. They were stained as in the previous series.

In the first stage of this series it is evident that there has been a large increase in the number of cells in the course of the olfactory nerve (fig. 6), but the distribution is the same as in the last stage of the previous series. The plane of section does not show the whole course of the olfactory nerve. The cells are more numerous peripherally but are entirely absent near the brain wall. The brain wall has raised up into a cone pointing into the olfactory nerve. This cone is an early stage of the olfactory bulb and its apex is devoid of cells, although there are numerous neuroblasts at its base where the mitral cells are forming. The mitral cells can be distinguished by their larger size and vesicular nuclei, aside from the fact that a wide fiber zone free of nuclei intervenes between them and the nuclei in the olfactory nerve. Consequently, there does not appear to be any evidence at this stage that cells are migrating from the brain into the olfactory nerve.

In sections of one or two of the fishes of the earliest stage of this series, there can be seen a slight aggregation of cells among the peripheral olfactory fibers at their anterior side (fig. 6, *a*).

This is the first indication of the ganglion of the nervus terminalis which, however, cannot be made out with certainty in some cases with specimens nine to twelve hours older. This indicates a rather wide range of variability with regard to its time of appearance. The cells of the aggregation at this early date are not greatly different from those elsewhere among the olfactory fibers. In other words, all of the cells along the olfactory nerve are in-

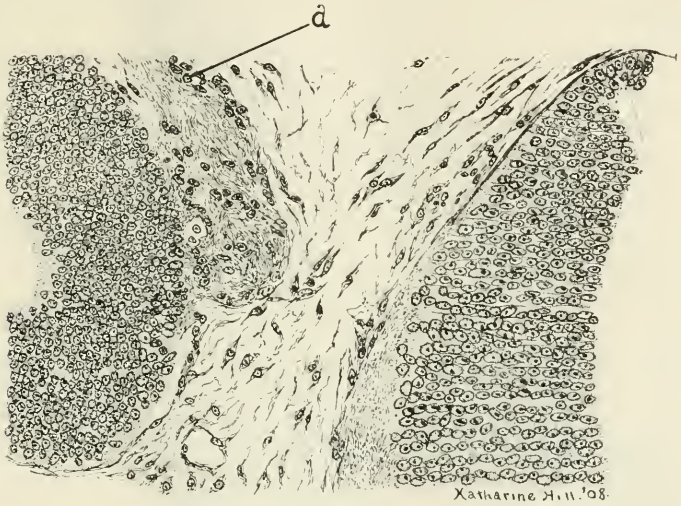

FIG. 6. Horizontal section of Amia during the second day after hatching. Shows the anterior end of the neural tube and part of the olfactory placode and nerve. Note the nuclei at *a* in the olfactory nerve near its origin from the placode. This is the point of origin of the cells of the nervus terminalis, not to be distinguished at this time from sheath cell nuclei. Iron hæmatoxylin and acid fuchsin. × 340.

different in character and still embryonic. Occasional mitoses have been found along the nerve in this and earlier stages. When the ganglion cells can be recognized they are slightly larger than the indifferent cells and their nuclei are more vesicular while their cytoplasm stains more deeply. Wherever the indifferent cells are taking on the characters of sheath cells, their nuclei have become elongated and stain more deeply than their

cytoplasm. On account of its deeper staining in hæmatoxylin preparations, the ganglion of the nervus terminalis is easily made out at a glance in stages two or three days older than this, i. e., in this series about five days after hatching (fig. 7, *a*). I was not able to recognize the ganglion until about ten days after hatching in another series preserved at longer intervals in

FIG. 7. Horizontal section of Amia about five days after hatching when the ganglion of the nervus terminalis is first recognizable. Note their position at *a* near the artery *b*. The olfactory nerve has two main rami to the nasal capsule *c* Olfactory bulb at *d*. Iron hæmatoxylin and eosin. × 340.

the previous year. This may be due to more rapid development during the warm weather of this present season. But the recognition of the ganglion at an earlier date may be due in part to better fixation and staining, as well as to the fact that the sections were cut horizontally so as to show the whole length of the

olfactory nerve and a greater surface of the ganglion, whereas the sections of the previously collected material were cut transversely. At all ages the ganglion appears more striking in sagittal and horizontal sections than in transverse, probably because the ganglion is slightly elongated in the direction of the axis of the body of the fish.

I have searched carefully for any placode or other source of origin of the nervus terminalis outside the olfactory capsule and nerve, but to no purpose, although Locy ('05) describes it as arising separately in Acanthias. If there is a separate placode in Amia, I have not been able to recognize it unless, indeed, the unpaired nasal placode already described is the beginning of the nervus terminalis, for this placode is the most anterior part of the nervous system I have found in Amia. In that event it would have to be said that the placode of the nervus terminalis is absorbed into the paired olfactory placodes from which the ganglia arise some days later.

Some extirpation experiments were made on very young Amia to determine, if possible, the mode of origin of the nervus te minalis. The nasal capsule was cut away from one side of the young, one or two days after hatching. The object was to remove the olfactory capsule completely without too much injury to adjacent parts. Probably complete removal was accomplished in only two cases out of over twenty operations, for there were some olfactory fibers found in all the nerves on which operations were performed except two. The fishes on which operations were made, were fixed and sectioned about a week or ten days after the extirpations. It is impossible to state to what extent regeneration of the olfactory nerve and placode took place in the short time the operated fishes were permitted to live, but in cases where one felt confident that all the placode was taken away as a more or less adherent mass of cells, there were found olfactory fibers later. It is probable that in all those cases where olfactory fibers were found on sectioning, a few cells of the olfactory placode were not removed in the operation. The number of fibers varies from a very few to half as many as were found on the uninjured side which was used for control. Along the fibers

were found sheath cells and a small ganglion of the nervus terminalis somewhat proportional to the size of the olfactory nerve as compared with the ganglion and olfactory nerve on the uninjured side.

Taking the inhalent nasal tube as a landmark, in some cases it appeared that more of the posterior part of the nasal placode had been removed and in other cases the anterior part. However, it did not seem to make any appreciable difference in the development of the ganglion whether the anterior or the posterior part of the nasal capsule was removed. From this fact we may infer that the ganglion of the nervus terminalis does not originate from any particular part of the olfactory placode. In the two cases where there were no olfactory fibers, no ganglion appeared, although small blood vessels were found near the brain and it is to be noted that the ganglion always appears near the blood vessels (fig. 7). From these experiments it appears, also, that the ganglion does not regenerate from the brain. The extirpation experiments taken by themselves do not absolutely prove that the ganglion does not arise from some other source near the olfactory placode, which source was disturbed in the operations that destroyed the placodes but, taken in conjunction with the embryological history of the ganglion previously given, they contribute some evidence to show that the ganglion is derived from the olfactory placode.

We may briefly sum up the early embryological history of the peripheral olfactory apparatus in Amia by saying that at a time about forty hours before hatching, two olfactory placodes exist in connection with what may be termed an unpaired olfactory placode. The latter disappears and the paired placodes immediately establish fibrous connection with the neural tube. Later, nuclei migrate out from the nasal sacs along the olfactory fibers as Disse ('96), and others have found for other forms of vertebrates. Still later some of the nuclei which have wandered out along the nerve form a ganglion, as Carpenter ('06) found for the ciliary ganglion in the chick, while others remain scattered among the olfactory fibers as sheath cells. In case of the ciliary ganglion, however, the nuclei migrate from the neural tube

along the developing oculomotor nerve, while in the case of the nervus terminalis the migration is from the placode toward the neural tube.

The nuclei of the ganglion on the olfactory nerve of Amia, can be distinguished readily from the nuclei of the sheath cells or of surrounding mesodermic elements when the young are about 10 mm. to 12 mm. long. These nuclei are larger than any others near them and are situated on the ventro-median side, as Allis, ('97) described them, about midway between the outer wall of the neural tube and the olfactory cup. There are perhaps two dozen nuclei on each olfactory nerve at this stage. The capsule which Allis describes as surrounding the ganglion has not been very evident to me, but there are smaller cells partly surrounding it on the side next the olfactory nerve, which I have taken for sheath cells of the olfactory nerve.

The ganglion increases in size and number of cells, but generally remains a single compact mass until about the 25 mm. to 30 mm. stage. In a few cases its cells have been found distributed in two or three aggregations along the olfactory nerve. It keeps its position about midway between the anterior end of the brain (bulbus olfactorius) and the most rostral part of the developing nasal capsule. The olfactory nerve is lengthening at this time and the brain lies farther caudad with respect to the eyes and some other structures when the fish has grown to maturity, and as Allis ('97) states, the olfactory nerve in the adult is about one and one half times as long as the brain. From the first appearance of the ganglion it is located near the point where a branch of the external carotid artery joins the olfactory nerve to be distributed to the nasal capsule.

When the young of Amia are from 25 mm. to 30 mm. in length, some of the cells, of which there are about forty at this stage, have wandered rostrad from the main ganglion and are scattered among the peripheral olfactory fibers. There are some five or six folds in the mucous membrane at this stage in each of two series lying one on either side of a median fold or "mid-rib" in each nasal capsule. Earlier the opening of each nasal capsule to the outside was a slit which has now closed in the middle to pro-

duce two apertures. One is larger and posterior near the eye, while the other is anterior and more median near the end of the snout and has already begun to develop a slightly protrusible nasal tube for the intaking of water. As the folds of Schneiderian membrane are smaller at the anterior end of the mucosa, it appears that new folds are added at this point as the animal grows. The cells from the ganglion scatter in a line beneath

FIG. 8. Golgi impregnation of part of the ganglion cells of the nervus terminalis in young Amia about 25 mm. long. A few olfactory fibers are shown. The latter are smaller and have slight varicosities. × 333.

the median fold or mid-rib. The cells are easily recognizable because their cytoplasm takes Delafield's hæmatoxylin more strongly than surrounding cells. That some of the ganglion cells are true nerve cells at this stage, is indicated by the fact that Golgi preparations (fig. 8) show branching processes,—toward the brain in this instance.

The 50 mm. stage is instructive and important, as the cells have attained practically the same distribution that they have

in the adult. In a section cut sagittally through the head and passing along the entire length of the olfactory nerve, the nasal capsule and its mid-rib, one can see the main ganglion and the scattered ganglion cells along the ventral side of the olfactory nerve (fig. 9). About two hundred and fifty cells on a single olfactory nerve were counted at this age. Nearly half of them are in the main ganglionic mass or near it, while the remainder are distributed almost uniformily from the ganglion rostrally to near the anterior end of the nasal capsule. They lie beneath the mid-rib rather than laterally under the secondary folds. There are some thirteen of these secondary folds on each side at this age, indicated by the number of undulations of the epithelium in the section (fig. 9). As the figure shows, the cells are practically all located between the anterior and the posterior

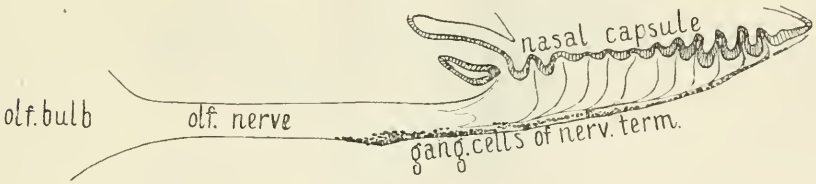

FIG. 9. Diagram from camera lucida outline to show the distribution of the ganglion cells of the nervus terminalis in young Amia 50 mm. long. Delafield's hæmatoxylin. × 20.

limits of the nasal capsule. One or two cells were found proximally of the ganglion along the ventral margin of the olfactory nerve, and two or three cells of the same appearance were seen ventrally near the point where the olfactory nerve joins the bulbus olfactorius.

In a series of Weigert sections cut transversely through the head of a fish whose total length was 100 mm. the ganglion cells along the olfactory nerve show quite well. Twenty cells were counted on the two sides along the proximal part of the olfactory nerves between the nasal capsules and the olfactory bulbs. Most of these cells lie within the cranial cavity. However, three

or four cells were found anterior to the cranial cavity at the level of the eye-muscle canal of Allis. They are located ventro-medianly between the two main rami of the olfactory nerve (fig. 10), but are more intimately associated with the median bundle. Fig. 10 may be taken as a typical cross-section of the olfactory nerve in this stage at any point anterior to the cranial cavity. In nearly every section across the region of the nasal capsules, from one to four ganglion cells may be found in the wedge between the two rami of the olfactory nerve. This is

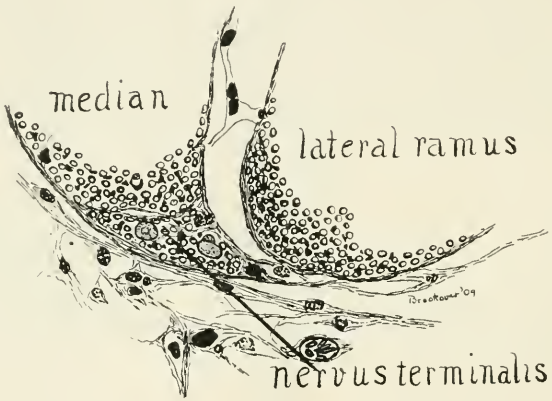

Fig. 10. Transverse section of olfactory nerve and nervus terminalis at level of the eye-muscle canal from Weigert preparation of young Amia 100 mm. long. Two ganglion cells show in the ventro-median wedge representing the nervus terminalis which is more intimately united with the median ramus of the olfactory nerve. × 210.

the portion which Allis ('97) denominated the nerve "*n*" of Pinkus. The fibers of this wedge, which is only occasionally slightly separated from the median bundle of the olfactory nerve by a few connective tissue fibers, show no trace of medullary substance. It appears similar to the olfactory nerve proper and cannot be traced in the series of sections except by its position and the presence of the ganglion cells. Allis has well said that there is interchange of fibers between the three bundles of the olfactory nerve.

When this ventro-median wedge is traced posteriorly into the cranial cavity it furnishes the fibers for a separate bundle (fig. 11) which passes to a more median position. This bundle has the ganglion cells already referred to, occasionally along its course, as figs. 11 and 12 show. However, there are variations with regards to the distinctness of the nervus terminalis in different cases as is well shown on the opposite, or left, side of this same fish where it is never more distinct within the cranial cavity than is indicated in fig. 10. Consequently, I was able to trace a nervus terminalis on the left side of this specimen, only by a more or less detached wedge of the median half of the olfactory

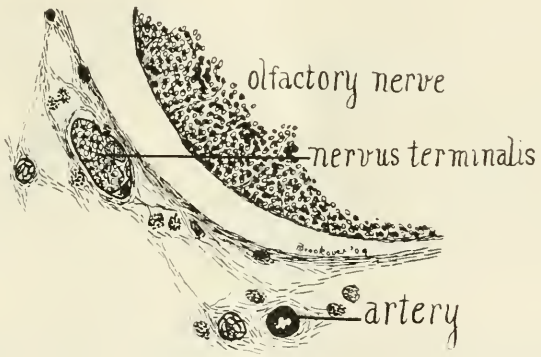

FIG. 11. From same series as fig. 10, but farther caudad within the cranial cavity. The nervus terminalis is distinct and shows a ganglion cell. × 210.

nerve, in which the characteristic large ganglion cells were found occasionally. It may be mentioned in this connection that I found a separate bundle of the left olfactory nerve in this fish, located on its dorso-lateral side at the level of the eye-muscle canal, but no ganglion cells were found along its course from the time it separated from the olfactory nerve until it reunited with it.

On the right side, the farther the nervus terminalis is traced caudad within the cranial cavity in this Weigert series, the farther it becomes separated from the main olfactory nerve (fig. 12). This figure is drawn at a level where it includes a large

ganglion cell outside the limits of the nervus terminalis and not far from a blood vessel. This section shows two other vessels which are the main branches of the internal carotid artery at this level. As the right nervus terminalis is followed caudad it becomes somewhat smaller and finally is lost in this preparation on account of poor fixation at this depth because the cranial cavity was not opened when the fish was killed and fixed. It will be shown later that the nervus terminalis joins the olfactory bulbs ventro-mesially and that the number of ganglion cells increases at this point in the adult.

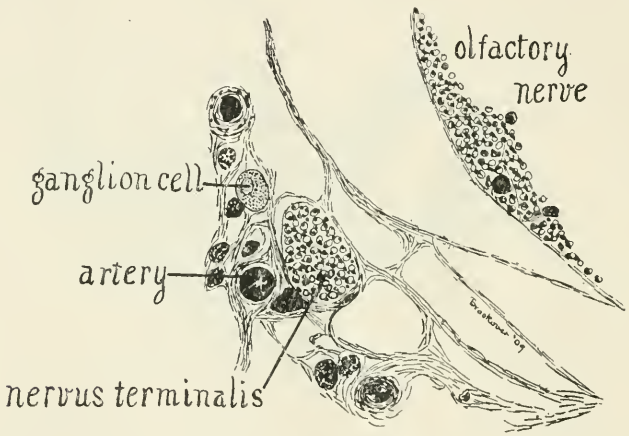

FIG. 12. From same series as figs. 10 and 11, but farther caudad within the cranial cavity near the olfactory bulbs. The blood vessels are quite near the nervus terminalis. A nerve cell is located between the nervus terminalis and the most dorsal vessel shown. × 340.

In the above mentioned Weigert preparations the blood corpuscles are stained a beautiful light blue, and since the blood vessels can be traced with great ease and they probably have the same distribution in the adult, they will be described here. Allis ('97) shows that the internal carotid artery enters the cranial cavity near the optic chiasm and sends a branch rostrad. As was said previously, this artery shows beneath each olfactory nerve intracranially (figs. 11, 12). Possibly it passes forward

beyond the cranial cavity to reach the nasal capsule, but this last point could not be determined with certainty from the preparation.

However, the main blood supply to the nasal capsules is derived from the external carotid artery. The branch which supplies each nasal capsule joins the olfactory nerve just anterior to the main mass of the ganglion of the nervus terminalis. This position of the artery with reference to the ganglion was found to be true for all stages of development subsequent to the appearance of the ganglion. The artery approaches the nerve from the ectal side and circles ventrally beneath it to come into close proximity with the ganglion. The artery divides as it runs forward and its branches passing near the scattered cells of the nervus terminalis, turn dorsad with the fibers of the olfactory nerve until they reach the basement membrane of the nasal epithelium lying along the mid-rib. From this point the arterioles turn laterally right and left, along each secondary fold of the Schneiderian membrane. An occasional nerve cell lies slightly dorsal of the olfactory nerve, but for the most part, the cells are located, as indicated in fig. 9, between the two main rami.

ADULT STRUCTURE OF THE OLFACTORY ORGAN IN AMIA

Amia lives much in swamps and Reighard ('03) has shown that when an adult is released from a boat it quickly buries itself in the slime at the bottom. The distensible nasal tube has a very small aperture which would be of advantage in filtering the water taken into the nasal capsules (fig. 13). The size of the nasal capsules, which almost equals that of a shark of the same size, indicate that Amia is macrosmatic as compared with other fishes. There are in the adults from fifty to seventy secondary folds on either side of the mid-rib of each nasal capsule (fig. 13).

Some experiments were made to determine how the water circulates in the nasal capsule. When colored fruit juice was introduced by means of a long pipette into the water of an aquarium containing a living adult, the nasal tube (fig. 13) inhaled

the juice. Later the colored fluid appeared at the exhalent aperture. In this experiment the current was intermittent and synchronous with the movement of the opercles in respiration, but there is also, a continuous egress of water between exhalations. This is probably due to the ciliary action to be described next.

In a pithed fish the nasal capsules were opened as shown in fig. 13, by removal of the nasal bone of Allis ('97, plate xx, fig. 1). When studied with a high power binocular dissecting microscope,

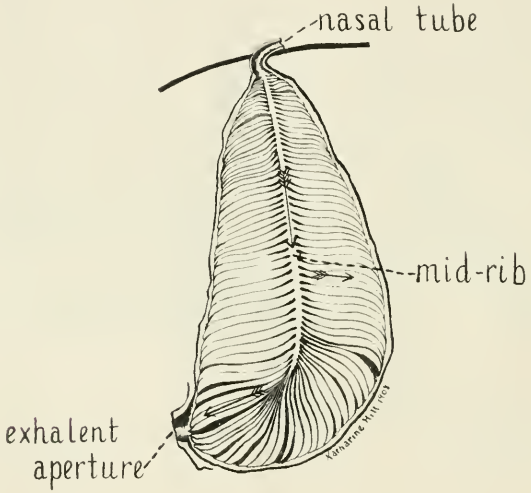

FIG. 13. Dorsal view of the opened nasal capsule of adult Amia to show the folds of the mucous membrane. The arrows show the direction of the water in passing through the nasal capsule. The water enters by the nasal tube, which is anterior. × 3.

the blood can be seen circulating laterally each way from the mid-rib along the dorsal margin of each of the secondary folds, as described previously for the young fish. The nasal capsules are very vascular and the circulation seen here reminds one of the circulation within the gills. When powdered carmine in water is introduced into the opened nasal capsule, it shows a current due to ciliary action directed posteriorly along the mid-rib and thence

laterally down between the secondary folds, as indicated by the arrows in the figure, to emerge at the sides of the capsules into a sort of drainage trough. From this trough the water can reach the posterior opening and pass to the outside. The mid-rib is at a lower level than the lateral ends of the secondary folds and some of the water passes posteriorly along the mid-rib and directly out between some larger folds there, or over them. A mucous substance is continually thrown out from the nasal epithelium and soon entangles the powdered carmine in ropy masses. This is probably produced by the goblet cells to be mentioned next.

Intra-vitam methylen blue and various cytological preparations show that there are in Amia three main types of cells (which have also been described by various workers on fishes) in the Schneiderian membrane, viz., olfactory cells of various shapes, ciliated supporting cells, and goblet cells secreting mucus. The ciliated cells are most numerous, while the olfactory cells come next in point of numbers. A ciliated supporting cell has a larger surface on the cavity of the nasal capsule than an olfactory cell. The mucous cells are isolated among the supporting cells and can be readily recognized in preparations stained with Delafield's hæmatoxylin.

In the adult fish there are only one or two important changes from the 50 mm. stage, in regard to the position of the ganglionic cells of the nervus terminalis. Most of the cells come to lie in the dorsal groove between the two main olfactory rami instead of ventrally, as in the early stages (fig. 9). This position is probably due to the increase of fibers in the lateral rami as the surface of the nasal folds to be supplied increases in area. But also, it may be that the cells migrate to a point nearer the place to be innervated. There are slight variations in the position of the cells in the adult. Sometimes a greater number of cells are found ventrally of the olfactory nerve than in others. In the adult there has ceased to be any decided ganglion at the posterior end of the nasal capsule. There are a few more cells posteriorly in the nasal capsule than farther rostrad, but the surface of the folds and the number of blood vessels are greater at the posterior end. The cells are nowhere aggregated in gan-

glia of any considerable size. Occasionally two or three cells may lie close enough to exert some mutual pressure on each other and flatten their adjacent sides (fig. 14), but generally they stand at some distance from each other. The figure was drawn from a slightly more crowded region than is typical in order to include as many cells as possible in one drawing.

Fig. 14. Ganglion cells of the nervus terminalis in a sagittal section of the olfactory nerve in adult nasal capsule of Amia. Taken from a slightly more crowded locality than is typical. Toluidin blue stain. × 333.

In a medium sized adult a little over one-third of a meter long, there were found to be nearly one thousand cells that were attributed to the nervus terminalis beneath a single olfactory capsule. The counting was done in sagittal sections of Nissl preparations, cut thick, and generally only such cells were counted as showed nuclei, in order to avoid counting a cell more than once. Fig. 14 is drawn from these preparations (toluidin blue stain), and shows

tigroid bodies and the large vesicular nuclei characteristic of functioning nerve cells.

More than twenty adult nasal capsules were treated by the Golgi method, but there were only two or three doubtful cases of impregnation of the ganglion cells of the nervus terminalis. There were many fibers shown in all of the preparations. The fibers belonging to these cells can be distinguished from those of the olfactory nerve (fila olfactoria) on account of their larger size and the fact that they often branch. Also, the olfactory fibers nearly everywhere show slight varicosities in their course (figs. 8 and 15), and are of lighter color, owing perhaps to their covering of sheath cells. The fibers of the nervus terminalis branch as they rise toward the mid-rib of the nasal epithelium (fig. 15), and were often seen following the course of the arteries

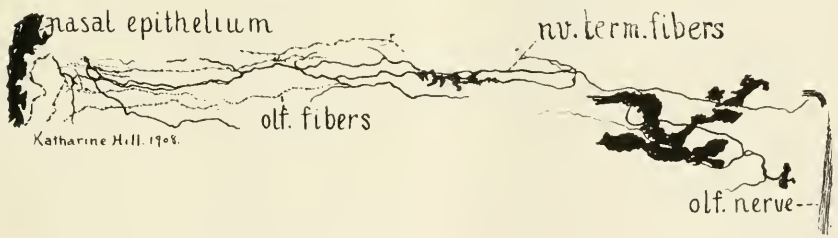

FIG. 15. Golgi preparation of the adult nasal capsule of Amia cut sagittally through the mid-rib. The coarse branching fibers belong to the nervus terminalis, the slender varicose fibers to the olfactory nerve. × 40.

(fig. 16). In no case were the branches followed into the epithelium of the nasal capsule, although careful search was made in both Golgi and intra-vitam methylen blue preparations where the finest end-branches were shown. In many cases they were followed into a reticulum of nerve fibers beneath the basement membrane of the epithelium (fig. 15). This reticulum is located at the point where the arterioles turn laterally along the secondary folds of the Schneiderian membrane.

Favorable preparations of the nasal capsules of adult Amia by the Cajal methods show the cells of the nervus terminalis, as well as the fibers related to them. For the reason that they show

these cells and have not in my hands inpregnated the olfactory fibers or cells, they are much preferable to Golgi preparations. In some cases neurofibrils are shown (fig. 17), thus indicating that they are functioning neurones in the adult fish. There are nerve processes ending on the surface of some of the cells or near them (fig. 17). It is difficult to say whether these are axones or dendrites. This is often the case in the symphathetic system, as for instance in the myenteric plexus (Auerbach's plexus) of the small intestine of mammals. In the case of the processes of

Fig. 16. Golgi preparation of adult Amia cut as above. Shows fibers of the nervus terminalis in the adult nasal capsule following an artery which is indicated by broken lines. × 80.

the nervus terminalis, they behave in the Cajal treatment like axones rather than dendrites. Cajal preparations and hæmatoxylin staining show that many of the cells are bipolar and spindle-shaped, but some of them are stellate with three or more processes. The third type comprising about one-half of the total number of cells, is fusiform in shape. So far as could be determined, these latter have only one nerve process that tapers gradually from the cell body like a dendrite. These are the three types of cells usually described in sympathetic ganglia.

CENTRAL RELATIONS OF THE NERVUS TERMINALIS IN AMIA

Near the olfactory bulbs each olfactory nerve breaks up into a large and variable number of small bundles. In Nissl preparations on one of the ventro-median bundles can be found cells of the same character as those found in the nervus terminalis in the nasal capsule. These cells (fig. 18) increase in numbers as the olfactory bulbs are approached. They are fusiform with the

FIG. 17. Fibers and cells from nervus terminalis in adult Amia by the Cajal method. Taken from the nasal capsules to show neurofibrils and fibers branching toward the periphery to end near the cells of the nervus terminalis. × 350.

pointed end generally directed toward the olfactory bulbs (figs. 18 and 19), while the opposite end is frequently capped with a cell of the same size and general appearance as the sheath cells. There are a few smaller ganglion cells, as fig. 18 shows. Nearly one hundred ganglion cells of this type were counted intracranially along the two olfactory nerves. I use olfactory nerve rather than nervus terminalis in the previous sentence because

I have never been able to satisfy myself that there are not olfactory fibers in this bundle even when separate. We have seen how it is sometimes united with the olfactory nerve in the 100 mm. stage, and the previous embryological account shows that it is developed as a part of the olfactory nerve.

Nissl preparations show that cells of the same general character as those just described, are continuous over upon the surface of the olfactory bulbs without any line of demarcation. Here they lie in groups of from two to as many as ten. They are situated outside the glomerular zone among the olfactory fibers and just inside a more or less continuous layer of neuroglia (fig. 21), from which they can be distinguished by their staining reactions in the Nissl preparations. Their size is larger than that of the supporting elements, but they are only about half as

Fig. 18. Cells in proximal part of nervus terminalis as it joins olfactory bulbs. Sagittal section showing the dendrites tapering proximally. Shows sheath cells characteristic of the olfactory nerve. Toluidin blue stain. × 340.

large as the mitral cells which lie at a deeper level beneath the glomerular zone. An estimate showed that there are at least two hundred cells that may confidently be said to belong to this category, on a single olfactory bulb. They are slightly more numerous on the mesial side of the bulbs but some were found in other positions as well. The majority of these cells appear to be bipolar. Catois ('01) has described such extra-glomerular cells in the olfactory bulbs of teleosts. Disse ('96) has found cells in Golgi preparations of embryo birds in the same position as those I have described in the olfactory nerve (nervus terminalis) of Amia where it joins the bulbs. Also, Rubaschkin ('03) has found peripheral cells in the frog's olfactory bulbs, which he denominates "sub-glomerular." Similar cells have been shown by Rubasch-

kin and Cajal to be in relation to the olfactory glomeruli. It may be that their endings in Amia have no connection with the nervus terminalus.

The course of the fibers of the nervus terminalis after joining the olfactory bulbs, was not made out with any degree of clearness until a large number of Golgi preparations were made of young Amia about 75 mm. long. One hundred of these small

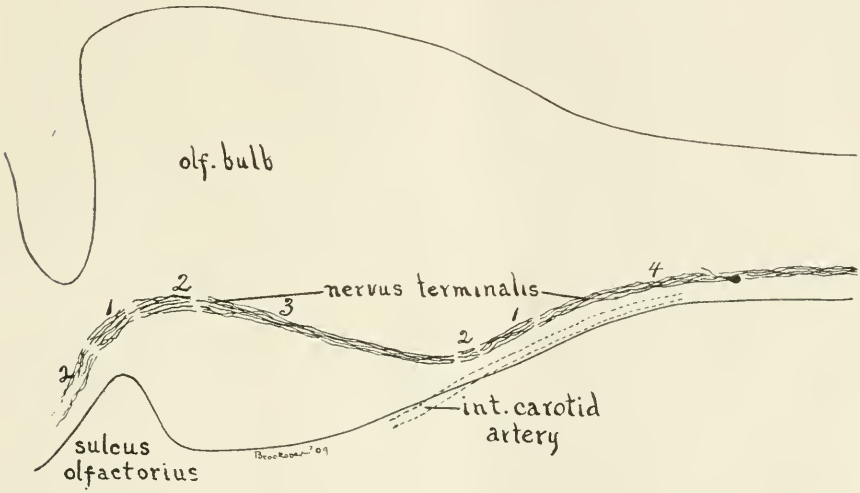

FIG. 19. Reconstruction from four sagittal Golgi sections 60 micra thick, of young Amia head, total length of fish 75 mm. All non-essential details omitted, to show the course of the nervus terminalis as seen from the median plane projected upon the outline of the most lateral of the four sections. The numbers indicate the sections from which a given part was taken. × 50.

fishes' heads were treated by the Golgi rapid process and transferred from the osmium-bichromate mixture to the silver in lots of ten or more at intervals of five or six hours from the second to the fifth day after immersion in the fixing fluid. In two of the earliest of these lots the fibers of the nervus terminalis were impregnated, while few or none of the fila olfactoria were shown. Fig. 19 is reconstructed from camera drawings of four sagittal

sections cut 60 micra thick and represents the nervus terminalis with all non-essential details omitted, as seen from the median side of the olfactory bulb and projected upon the deepest or most lateral of the four adjacent sections. This is one of more than a dozen fishes that show essentially the same thing.

The above preparations show the nervus terminalis with varying degrees of distinctness from the region of the main ganglion of cells rostrad of the eye-muscle canal along the ventro-median edge of the olfactory nerve until its fibers join the olfactory bulb. A very few impregnated cells have been seen in my preparations (figs. 19, *4*), but that this bundle is the nervus terminalis rather than fila olfactoria, is shown by the following facts: it is composed of slightly coarser fibers than the fila olfactoria, arises from the ventro-median part of the olfactory nerve, turns ventro-caudad over the median surface of the anterior one-third of the olfactory bulbs, then caudad, and finally ventro-caudad into the prosencephalon proper. Some of its fibers may end in the olfactory bulbs, but a number of them continue into the forebrain.

The course of the nervus terminalis as seen in horizontal sections is shown in fig. 20. This figure was reconstructed from Golgi preparations in the same way as fig. 19. Fig. 20 shows the nervus terminalis as seen from the ventral side of the brain, projected upon the outline of the most dorsal of the five sections showing the nervus terminalis. In consequence of the more ventral sections of the olfactory bulbs being smaller in area and the fact that the mass of the bulbs was slightly shrunken and contracted away from the median surface of the olfactory bulbs, the nervus terminalis appears more deeply embedded in the bulbs than is really the case. The real depth of the nervus terminalis is more accurately shown by fig. 21 which is taken from a Cajal preparation of the median side of the adult olfactory bulb at about the middle of its antero-posterior extent. Also, fig. 21 shows the cells previously mentioned as of a different nature from the mitral cells, and as being more superficial in position. In one or two Golgi preparations I have found some evidence that fibers believed to belong to the nervus terminalis end in relation to cells on the surface of the olfactory bulbs medianly.

The maximum number of fibers of the nervus terminalis impregnated in any one of the Golgi preparations of fishes 75 mm. long has not exceeded twenty. One cannot say whether these are all of the fibers that belong to the nervus terminalis, but often

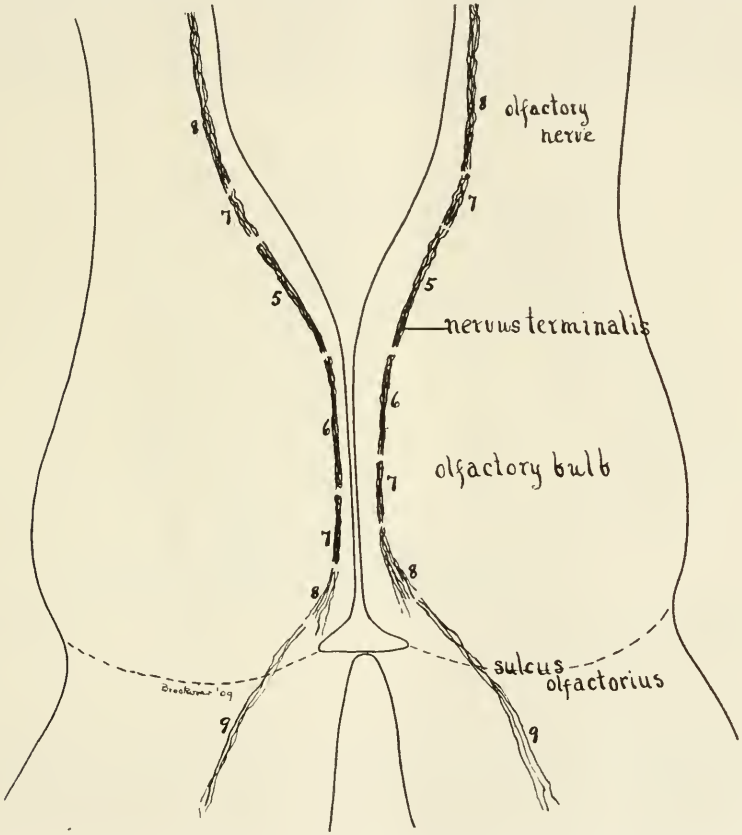

FIG. 20. Golgi preparation from a young Amia about 75 mm. long. Reconstructed from camera lucida drawings as in fig. 19, but taken from horizontal sections and shows the nervus terminalis as seen from the ventral side of the olfactory bulbs projected upon the most dorsal of the sections. × 50.

the Golgi process impregnates a majority of the fibers of a given kind. My impression from all the preparations and the difficulty with which the fibers were found in any of the preparations of young or adult, is that there are not more than about twenty-

five fibers in the nervus terminalis at this age at the point where it joins the olfactory bulb, although there are not less than two hundred and fifty ganglion cells peripherally at this time. The maximum number of fibers at this point in the adult as shown by the Cajal process, which is supposed to show all the fibers of a given kind, did not exceed forty. Herrick ('09) and Sheldon ('09) traced the nervus terminalis posteriorly into the anterior

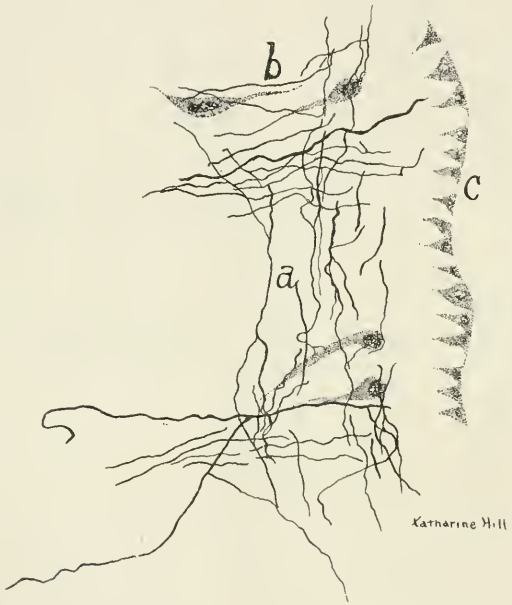

FIG. 21. Cajal preparation cut perpendicularly to the median surface of the olfactory bulbs of adult Amia, 15 micra thick. Shows what were thought to be fibers of the nervus terminalis at *a*, and ganglion cells at *b*. Supporting elements on the median surface of the bulbs at *c*. × 444.

commissure, but the bundle is diffuse in Amia and has not been traced into the commissure as yet.

As noted in an early part of this paper, Allis traced a root of the nervus terminalis posteriorly ventral of the prosencephalon to the region of the optic chiasm, but I failed to find it in gross dissections. However, I have frequently found a bundle of non-medullated fibers accompanying the internal carotid artery of each

side in this position. Fig. 22 taken from a single sagittal section of a Golgi preparation of a young Amia 75 mm. long shows the relation of the nerve fibers to the artery at the anterior end of the olfactory bulbs in the position where the internal carotid artery is shown in fig. 19. It will be noted (fig. 22) that a fairly compact bundle of five or six fibers accompanies the main artery. From near it two fibers run dorsad along a blood vessel. The

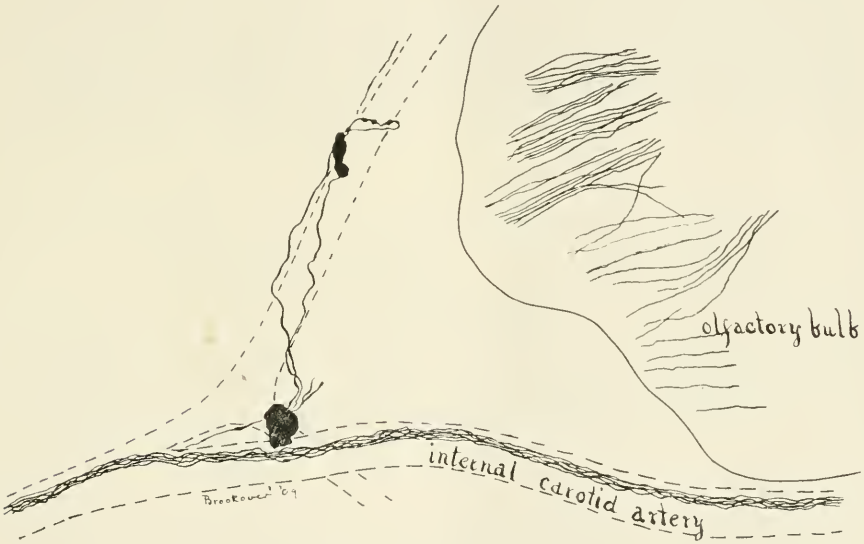

Fig. 22. Sagittal section in the median plane from a Golgi preparation of a young Amia about 75 mm. long. Shows a bundle of about five fibers following the course of the internal carotid artery at the level of the anterior end of the olfactory bulbs. The fibers become mingled with those of the olfactory nerve in the region of the nervus terminalis, in the adjacent section. × 225.

main bundle, which can be followed from posterior to the sulcus olfactorius between olfactory bulb and hemisphere, runs forward beyond the olfactory bulbs to be lost among the fibers of the olfactory nerve. I have found fibers behaving like those that run dorsad in fig. 22 in Cajal preparations accompanying the small arteries among the bundles of the olfactory nerve in adults at the level of the anterior part of fig. 19. Also, I have

more than once found fibers accompanying the blood vessels that run into the adipose tissue of the cranial cavity rostrad of the olfactory bulbs.

In Cajal preparations of adults I was able to trace a bundle of fibers, not exceeding fifteen in number, from the olfactory bulbs posteriorly to the region of the optic chiasm, in the same relation to the internal carotid artery as shown in the Golgi preparations just mentioned (see also fig. 32). In one Cajal preparation the fibers seemed to diminish in numbers posteriorly, but I was not able to connect this bundle with the nervus terminalis on account of a defect in the preparation, as I judged, although I succeeded in tracing the bundle within less than a millimeter of

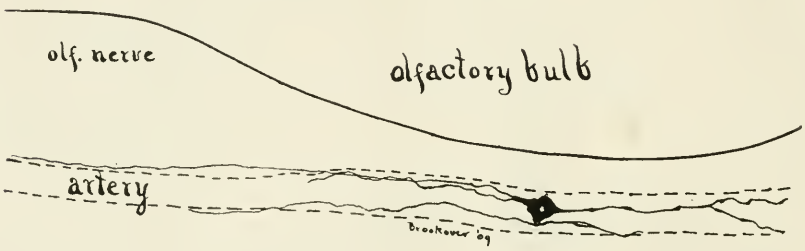

FIG. 23. Golgi preparation from young Amia as for previous figure. Shows what was thought to be a nerve cell beneath the olfactory bulb, the most posterior of any cells in the meninges attributable to the nervus terminalis. × 225.

the bundle of the olfactory nerve containing nervus terminalis fibers.

What was thought to be a nerve cell with its processes branching in the neighborhood of the internal carotid artery beneath the olfactory bulbs (fig. 23) was found as far as posteriorly as the bit of artery shown in fig. 19. This is the farthest caudad that a cell separated from the main branch of the nervus terminalis has been found within the cranial cavity, except the groups of nerve cells to be described later. The position of the nerve cell in this instance (fig. 23) appears quite similar to that of the

undoubted nerve cell shown in fig. 12 near the nervus terminalis. Golgi preparations of adult Amia show that there are branching nerve fibers along the arteries at the anterior end of the olfactory bulbs (fig. 24), which are apparently derived from the nervus terminalis. They are more numerous ventral of the olfactory bulbs, but they have been found on every side of the bulbs and oftentimes seem to arise from the bulbs or are following the vessels to the very surface of the bulbs. There is a rich network of vessels at the surface of the olfactory bulbs and injected Nissl preparations, as well as Golgi impregnations, show that every large mitral cell has an arteriole coursing along the surface of its two or three main dendrites.

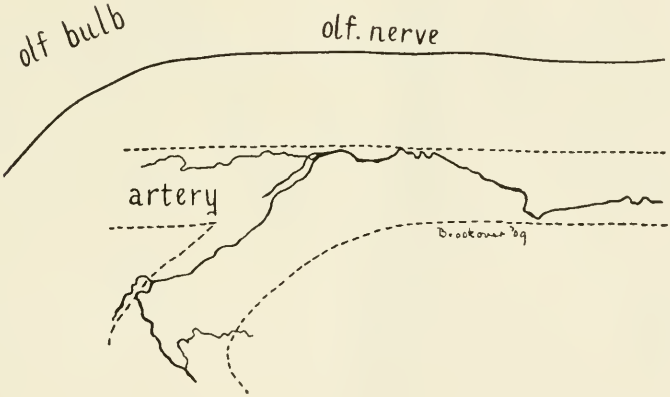

FIG. 24. Golgi preparation cut sagittally from adult Amia. Shows nerve fibers proceeding posteriorly from the position of the nervus terminalis, near where it joins the olfactory bulb ventro-mesially. × 225.

From the above description, from the findings of Allis, and from the fact that Pinkus found the nervus terminalis running far caudad ventral of the brain in Protopterus, it would not be unnatural to infer that the nervus terminalis in Amia extends caudad of the olfactory bulbs. However, we shall be in a better position to judge of this matter after a description of certain fibers found at all levels in the meninges of the prosencephalon of Amia.

INTRA-CRANIAL SYMPATHETIC SYSTEM POSTERIOR TO THE NERVUS TERMINALIS IN AMIA

In addition to the fibers already described as occurring in the cranial cavity on the blood vessels, there were found in numerous Cajal and Golgi preparations a number of fibers among the blood vessels and glandular tubes of the paraphysis. These fibers could often be traced into a more or less distinct bundle near the posterior lateral portion of the paraphysis. This is just outside the brain membranes near the anterior edge of the optic tracts. As Huber ('99) had found nerves entering the cranial cavity of mammals along with the internal carotid artery near the optic chiasm, search was made for a long time to discover the entrance of fibers at this point in Amia, but to no purpose. Nerve fibers were found entering the cranial cavity dorsally opposite the anterior end of the epiphysis (fig. 25) through a foramen by which a vein apparently leaves the cranial cavity. This foramen is near the alisphenoid ossification (Allis, '97) and is probably the one marked "foramen for the anterior cerebral vein" (plate xxi). In Weigert preparations of the head of a young Amia 100 mm. long there are seen to be five or six medullated nerve fibers entering at this point. There are probably some non-medullated ones as well, From dissections of adults there were found to be three rami of this bundle diverging from a point just inside the cranial cavity (fig. 25). The first ramus runs mesially at right angles to the long axis of the body to a point near the anterior end of the stalk of the epiphysis. A second branch runs forward to be lost in gross dissections in the fat surrounding the brain. The third and largest ramus turns posteriorly to a point just in front of the habenular body and is distributed forward among the tubules of the paraphysis, as already mentioned. Some fibers are sent ventrally along the arteries to the neighborhood of the hypophysis, where nerve fibers have been found on the blood vessels. Gross dissections show some variations in size and distribution of these rami in different specimens.

In one adult Amia there were found more than thirty large nerve cells along the intra-cranial part of these rami. About

half of them were situated near the entrance of the nerve into the cranial cavity (at point *a* in fig. 25), while the remainder were found along the posterior branch of the nerve near the paraphysis and the pallial wall of the forebrain (at point *b* in fig. 25). The latter ganglion is situated in close proximity to the base of the

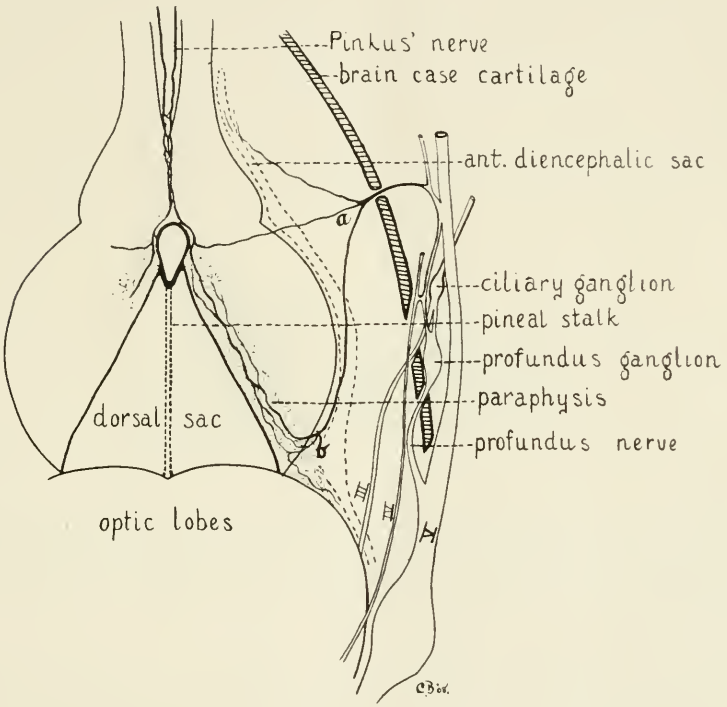

FIG. 25. Diagram showing dorsal view of the anterior end of adult Amia brain. Made from gross dissections with the aid of Weigert preparations of a young Amia 100 mm. long, to show the relation of the profundus nerve to the ramus which innervates the meninges of the forebrain. Also, it shows the relation of the preoptic sympathetic system to the pineal nerve and to the nervus terminalis. × 4.

dorsal sac (fig. 30) and near the origin of the diencephalic sacs of Kingsbury ('97), from the third ventricle. In total mounts of the intra-cranial part of the nerve, sheath cells can be seen surrounding the large nerve cells (fig. 26). The cells show tigroid bodies and are of the same size and character as those of the pro-

fundus nerve outside the cranial cavity. However, some of the cells are only half as large as others and the smallest are about the size of the cells of the nervus terminalis.

The fibers that enter the cranial cavity arise from the ramus ophthalmicus superficialis trigemini where the latter is joined by a portion of the profundus nerve as it extends forward from its main ganglion (Allis, '97, plate xxx, fig. 39, *opt*). From Weigert sections it seems pretty certain that most, if not all, of the fibers that enter the cranial cavity arise from the profundus nerve. As the profundus nerve furnishes a connection between the posterior part of the sympathetic system and the ciliary ganglion, it may well establish a connection from the sympathetic system

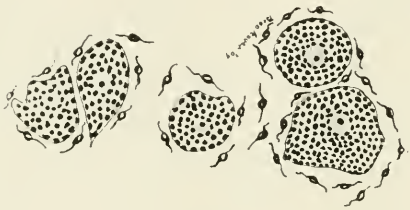

FIG. 26. A portion of the ganglion cells from point *b* in fig. 25. Camera drawing from the most crowded portion of the ganglion, the meninges being mounted without sectioning. Shows sheath cells around the nerve cells and Nissl bodies within. × 200.

to the cells inside the cranial cavity, and to the nervus terminalis. It may be said here, that there are nerve cells along the profundus nerve up to the point where it joins the ramus ophthalmicus superficialis trigemini and sends its fibers into the cranial cavity. Also, in Weigert preparations the profundus nerve was found to send fibers into the trochlear nerve as it passes near (fig. 25).

An interesting question arises as to the origin of the nerve cells within the cranial cavity of adult Amia. Total mounts of the nerve did not always show the cells and when found, they are quite variable in number. Moreover, it appears that the nerve fibers as well as the cells are sometimes asymmetrical as regards the two sides. In the cases noted there was greater development

on the right side of the specimen. As the cells are very much like those of the profundus nerve outside the cranial cavity, it was at first inferred that they migrate into the cranial cavity along the fibers from the profundus nerve. This seemed all the more probable because the cells inside the cranial cavity were not found in sections of young specimens, but the number of cells is very small in the adult and might be overlooked very easily in the young among so many blood vessels. There is an interesting observation to be mentioned here as having a possible bearing on the origin of the posterior group of cells within the cranial cavity. This group lies not far from the position of the evanescent thalamic nerve described by Miss Platt ('91) in Acanthias. In fact, in one Cajal preparation of adult Amia, it was thought that a few fibers of the thalamic nerve were found entering the brain laterally anterior to the habenular bodies. For some reason there was a break in the continuity of the fibers at the brain wall. Inside the brain, fibers were seen running from this point. As no evidence of a nerve in this location has been found in any other specimen the presence of a thalamic nerve in Amia is in doubt.

When the nerve cells at this point (*b* in fig. 25) are examined in surface views of total mounts, they are found to be well scattered. Fig. 26 shows five cells in their natural relation to one another in the most crowded portion of the ganglion of nearly twenty cells. Some of these cells are closely applied to the membranous pallial wall of the forebrain. In Cajal preparations I have found one or two nerve cells lying near the median line among the paraphysis tubes situated between the dorsal sac and the pallium of the forebrain. Fibers were often found here among the paraphysis tubes establishing what may be considered as a commissure between the two halves of the intra-cranial sympathetic system.

As already mentioned, Golgi impregnations show that the blood vessels everywhere within the cranial cavity have nerve fibers branching on their walls, but it is not so easy to determine whether the paraphysis and the ciliated epithelium of the dorsal and diencephalic sacs have their intrinsic nerves or not. The paraphysis is a glandular structure with tubes gathering to a duct which pours its secretion into the brain ventricle just beneath the middle

of the pineal stalk (fig. 30). Kingsbury ('97, *a*, fig. 4) shows the opening of to the duct of the larval paraphysis. There are many instances in Golgi impregnations that seem to me to show that there is an intrinsic nerve supply to its tubes (fig. 27). Fibers often lie in the closest proximity to the blood vessels on one side and to paraphysis tubes on the other. Also, fibers are occasionally found between the tubes of the paraphysis and the ciliated epithelium of the pallium (fig. 27). In the basement membrane of the dorsal and diencephalic sacs there are often found nerve fibers in the same intimate relation with the epithelium on one side and the

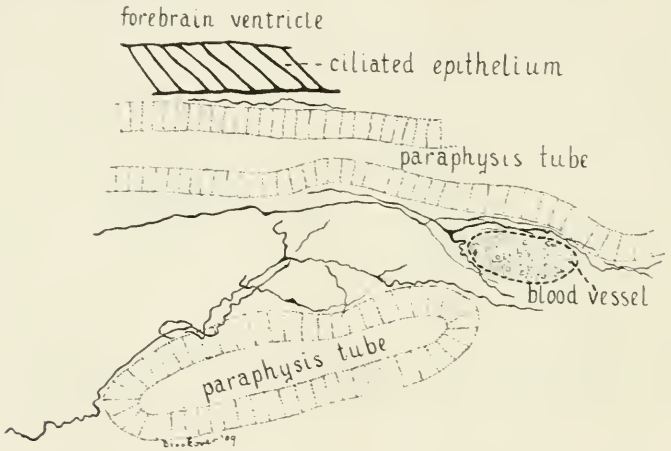

FIG. 27. Golgi preparation of the meninges of adult Amia showing the relation of the nerve fibers to the paraphysis tubes and to the blood vessels near the pallial covering of the forebrain. × 200.

blood vessels on the other. The richest supply of nerves is found among the paraphysis tubes to which the majority of the medullated fibers entering the cranial cavity were traced. The next richest supply of nerves is furnished to the blood vessles near the ciliated pallial epithelium, but some fibers are found on the walls of the blood vessels in all positions in the cranial cavity.

The ciliated epithelium just mentioned merits a closer examination into its structure and function. It may be said at the outset that there is the same essential structure of the pallium of the

forebrain, of the dorsal sac, and of that side of the diencephalic sacs farthest from the brain wall. The ental side (toward the brain) of the diencephalic sacs is made up of delicate flattened epithelial cells, as Kingsbury ('97, *a*) has noted. Also, he has pointed out the fact that the high columnar epithelial cells of the pallium and diencephalic diverticula are glandular in appearance and that they have a more copious blood supply than the flat cells on the ental side of the diencephalic sacs.

Favorable fixations and staining in iron hæmatoxylin show that these columnar cells are ciliated (fig. 28) with from three to six long stout cilia to each cell. Portions of the live epithelium mounted in normal saline solution show that the cilia are active in producing motion in the encephalic fluid. Strips of the epithe-

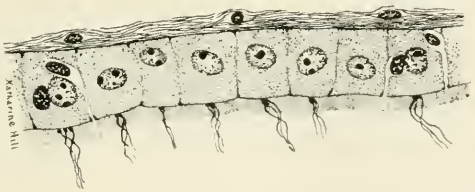

FIG. 28. Section of the membranous pallium of the forebrain of adult Amia. Shows the motile cilia, the granular contents of the columnar epithelium and its cuticular border. Two "mast" or wander cells are shown between the cells of the epithelium proper. Iron hæmatoxylin stain. × 444.

lium running parallel with the long axis of the brain show by the motion of the blood corpuscles which have escaped from the vessels, that the general direction is anterior in the common forebrain ventricle. Strips cut from the ventricular walls of the forebrain showed that there is ciliary action by its cells also, producing motion rostrad along the slit between the halves of the prosencephalon. Likewise, there is ciliary action on the walls of the rhinocœls. The few experiments made seem to show that the return path of the encephalic fluid runs laterally from the rhinocœls and posteriorly along the lateral everted portion (Kappers '07) of the forebrain. The cilia have basal bodies and the free borders of the epithelial cells possess a cuticula which is striated

perpendicularly to the surface of the cells so as to make them appear as if there were a second set of shorter cilia. The contents of the cells are granular with large nuclei located slightly deeper than the center of the cell. Frequently there appear to be two or three nucleoli. There are vacuoles in the cytoplasm in certain preparations, but not enough work has been done on the finer structure of the cells to determine whether fat or other substances have been dissolved in the treatment with alcohol or not. In preparations stained with intra-vitam methylen blue or by the Nissl method, there appeared certain cells occasionally, that were a deeper blue than the majority of the columnar cells. They were brought out in some of the iron hæmatoxylin preparations in which they seemed not quite so granular as the other epithelial cells. They are closely applied to the basement membrane from which they taper to a narrow end at the free surface. When the epithelium is viewed from its deeper surface, these cells show radiating arms that seem almost to set them in connection with one another. Thus they look very much as if they form a nerve plexus. These cells call to mind the supporting cells which Johnston ('01) described among the ciliated epithelial cells of the saccus vasculosus in Acipenser.

Our knowledge of the structure and function of the diverticula of the neural tube of vertebrates is not very extensive. Meek ('07) has studied the choroid plexus of the lateral ventricles of some mammals and has shown that there is a single layer of cubical cells in the epithellium. These are ciliated in the young but devoid of cilia in the adult. He found motile cilia in the adult on the ependymal walls of the ventricles. He shows that nerves are present in close proximity to the blood vessels and that the epithelium sometimes has intra-cellular fat globules. There is sometimes a cuticular border on the free surface of the epithelial cells of the choroid plexus that, as in Amia, gives them the appearance of being ciliated. Johnston found nerve fibers in the basement membrane of the ciliated epithelium of the saccus vasculosus in Acipenser and, as we have seen that cilia are present and cause motion in the encephalic fluid in Amia where there are nerve fibers in the basement membrane, we might infer that cilia are con-

trolled by nerves. However, Pütter ('03) states that it has not been proven that ciliary action is under the control of the nervous system except perhaps in a few molluscs and annelids.

The pineal stalk of Amia has an innervation that shows some characteristics of sympathetic nerves. Cajal preparations show that there are more than fifty nerve fibers joining the brain from the pineal stalk. Some of these fibers pass to the habenulæ, but a larger number turn caudad to the region of a gland-like structure beneath the superior commissure. Johnston ('01, p. 108) has called this structure in Acipenser the epiphysial sac and shows that nerve fibers end among its cells but he tells me these fibers do not come from the epiphysis. It is prominent in many preparations of Amia since it stains deeply with methylen blue

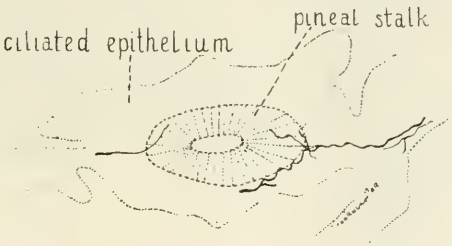

FIG. 29. From the same preparation as fig. 27, showing the relation of nerves of the meninges to the pineal stalk. × 64.

and with hæmatoxylin. Most of the nerve fibers from the pineal stalk are soon lost in all of my preparations near the walls of the third ventricle, after passing the glandular epiphysial sac.

In a fortunate Golgi preparation of a young Amia about 12 mm. long I was able to show the details of a neurone of the pineal stalk (fig. 31), in a single section. This neurone has its cell body in a position near the distal end of the stalk. Its dendrites branch near the surface of the stalk, while its axone passes centrally to be lost between the habenula and the superior commissure. At this time the pineal stalk comes into contact with the ectoderm and I have often noticed in very young specimens of about this same size that there is a light spot in the skin free of

pigment in this location. However, as the young fish grows, bone and cartilage intervene between the skin and the pineal stalk. The distal end of the pineal stalk in the adult fish is slightly enlarged (fig. 30) and adheres to the cartilage on the roof of the brain case.

The nerve fibers and cells of the adult pineal stalk of Amia were found to be particularly susceptible to methylen blue used intra-

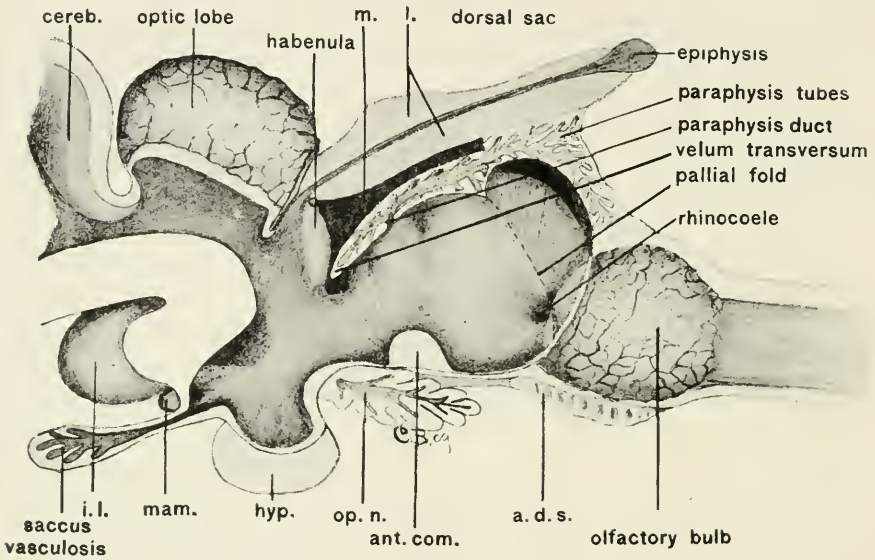

FIG. 30. View of the left half of the anterior part of the adult brain of Amia as seen from the median plane, to show the relation of the pineal stalk, the paraphysis, and the diencephalic sacs to the brain ventricles. Partially schematic from camera lucida outlines. × 6. *a. d. s.*, anterior diencephalic sac; *ant. com.*, anterior commissure; *cereb.*, cerebellum; *hyp.*, hypophysis; *i. l.*, inferior lobe; *mam.*, mammillary body; *m. l. dorsal sac*, median and lateral portions of dorsal sac; *op. n.*, optic nerve.

vitam. A number of fine impregnations were procured by injecting methylen blue through the ventral aorta just anterior to the heart. Fig. 33 shows a drawing made from such a preparation mounted without sectioning and represents the pineal stalk at about the middle of its length. The longitudinal nerve fibers already mentioned as occurring in Golgi and Cajal prepara-

tions, are shown in numbers, with nerve cells at intervals. The nerve cells have from one to four branching processes establishing anastomoses which set the longitudinal fibers and the cells in connection with each other. When the cells are examined with a high magnification there does not seem to be any marked differentiation into axones and dendrites (figs. 34 and 35). All the cell processes look quite similar, as is the case in an intestinal sympathetic plexus. Moreover, the fibers appear to be continuous from one cell to another. The cytoplasm of some of the cells shows a copious supply of tigroid bodies (figs. 34

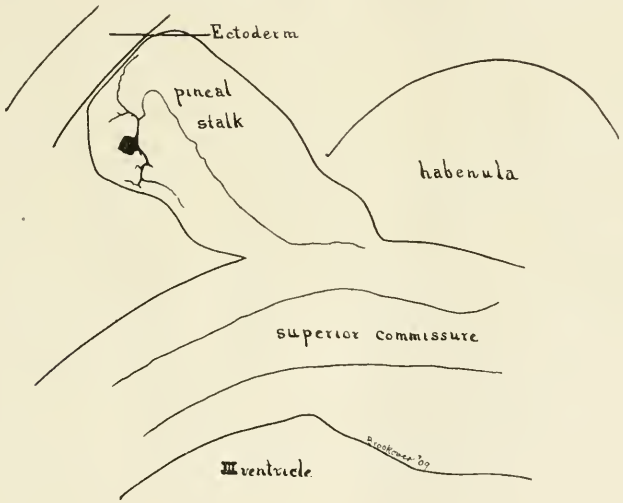

FIG. 31. Approximately transverse section of a Golgi preparation of young Amia about 12 mm. long, to show the details of a neurone of the pineal stalk. × 200.

and 35). There is an epithelium lining the tube of the pineal stalk. The nerve cells and fibers lie among the bases of these epithelial cells near the basement membrane. The structure of this epithelium has not been studied to learn whether it shows evidence of being glandular or not, but I have noticed a set of capillaries here with finer meshes. The enlarged distal end of the stalk has essentially the same structure and manner of innervation as the stalk proper.

In fig. 33 there is a transverse fiber that runs across the stalk of the epiphysis at a higher level than the longitudinal fibers and appears to leave the confines of the pineal stalk. This seems to provide for connection with the nerves already described among the tubes of the paraphysis. Also, in a number of Golgi impregnations the fibers of the nerves of the meninges reach the basement membrane of the pineal stalk (fig. 29), if indeed, they do not pass into it. This provides a nervous mechanism capable of correlating the blood supply or the fluid secretions of the paraphysis and the pineal stalk.

In the diagram of the intra-cranial course of the sympathetic system of Amia (fig. 25), I have connected the fibers from the profundus nerve with the pineal stalk on the above evidence. The pineal structures of vertebrates are generally considered atavistic remnants of a former eye. If such an eye had a sympathetic component, the nerve supply to its stalk in Amia might perhaps be considered as remaining after sight degenerated. Or there might have been a complete change of function resulting in the present nervous structure of the pineal stalk. However that may be, it can be seen (fig. 25) that there is provision for a longitudinal connection between the supposed ancient nerve, the hypothetical thalamic, and the post-optic sympathetic system. It should be explained in this connection that point "*b*" is anterior to point *a* (fig. 25) in young specimens of Amia so that the sympathetic chain runs forward instead of bending posteriorly. The brain is carried farther caudad in its cavity as the fish comes to maturity. The above described sympathetic chain furnishes connections with the posterior part of the sympathetic system for two of the three supposed neuromeres which Johnston ('05) and others have assigned to the forebrain. The last link in the forward extension of the sympathetic chain is probably represented in the connection of the intra-cranial sympathetic just described with the nervous terminalis, now to be considered more fully than before.

In discussing the central connections of the nervus terminalis I have pointed out the fact that non-medullated fibers were found along the arteries ventral of the olfactory bulbs and that this

bundle of fibers passes caudad of the sulcus olfactorius. Fig. 32 shows a reconstruction from nine horizontal Golgi sections drawn with the aid of a camera lucida. The same essential facts are shown by a number of other Golgi preparations of fishes 75 mm. long which belong to the same lot of fishes previously mentioned as showing the nervus terminalis favorably. On the

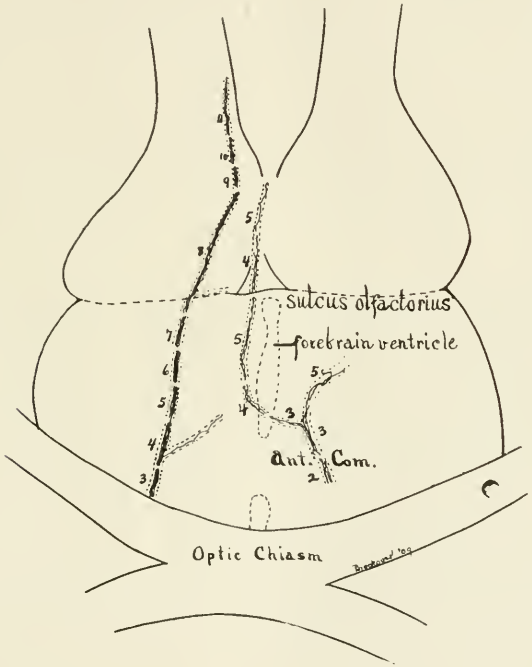

Fig. 32. Reconstruction from ten camera lucida drawings of consecutive sections of Golgi preparations of young Amia 75 mm. long. Shows the course of the fibers following the internal carotid artery from the optic chiasm to the region of the nervus terminalis. Arteries indicated in broken lines. The numbers indicate the section from which parts were taken. × 25.

left side, which is more fully impregnated (fig. 32), a bundle of fibers follows the main branch of the internal carotid artery from the region of the optic chiasm to the region of the nervus terminalis. The arteries are plainly visible in the preparations and are shown by broken lines in the figure. The main bundle of fibers

impregnated does not show more than five or six fibers, and there is slight diminution in numbers forward. As we have seen (fig. 22), there may be as many as six fibers impregnated at this age at the level of the anterior end of the olfactory bulbs. It will be noted (fig. 32) that a lateral branch is given off medianly at the level of the anterior edge of the anterior commissure which is drawn in position by the aid of the camera lucida. There is always a pair of arteries entering the forebrain at the level of the anterior edge of the anterior commissure and in one instance in Cajal preparations of adults I thought I found a fiber entering the brain along with the artery, but this is the only instance I have ever been able to find of fibers entering the ventral surface of the brain between the posterior part of the olfactory bulbs and the optic chiasm, although I have searched carefully in all of my preparations.

On the opposite side (fig. 32) fibers reach the same point near the anterior commissure and continue forward along the blood vessels near the mid-line as far as the anterior median margin of the olfactory bulbs. At the level of the sulcus olfactorius the internal carotids always give off one or more branches medianly. These branches turn dorsad in the fold of pallium which Kappers ('07) shows as separating the common forebrain ventricle into lateral halves at its anterior end (fig. 30). In a fish which had its medulla oblongata and spinal cord pithed, I have watched the blood circulating dorsad in this region to the neighborhood of the anterior end of the pineal stalk (fig. 30). In Cajal preparations I had traced fibers along the course of these arteries from a compact bundle of six or eight fibers at the level of the posterior ventral margin of the olfactory bulbs until they scattered beneath the paraphysis (fig. 30). Finally in three out of four Golgi preparations of adults made at one time, I confirmed my findings in Cajal sections and came to regard the fibers in this position as a constant feature. They are non-medullated and their maximum number does not exceed twelve in adult Amia. Almost invariably when the conditions have been favorable for impregnation of these fibers in their protected position they have failed to impregnate in Golgi and Cajal preparations the fibers in the

region of the posterior ventral margin of the olfactory bulbs. If one had found the nervus terminalis but had failed to find the intra-cranial sympathetic fibers posterior of the olfactory bulbs, he might have supposed that the fibers in the fold of the membranous pallium between the forebrain and in the meninges ventrally were roots of the nervus terminalis as Allis apparently did, but I have satisfied myself from my preparations that the bundle of fibers in the median fold of the membranous pallium is a part of the system of fibers innervating the meninges of the fore-brain (figs. 22 and 32.)

In concluding the description of the intra-cranial sympathetic fibers of the meninges of the forebrain of Amia, it can be said that there is ample opportunity for connection between the nervus terminalis and the post-optic sympathetic system. There is a constant bundle of about six non-medullated fibers that can be traced in fishes 75 mm. long from the optic chiasm to the region of the nervus terminalis (fig. 32). Cajal preparations of the adult show that there may be three times as many fibers in this bundle at maturity. This bundle has not been traced into the brain near the optic chiasm as Allis ('97) seemed to think might be the case, but in many preparations I have connected it by a bundle of fibers following the blood vessels dorsad, with the fibers entering the cranial cavity from the profundus nerve (fig. 25). The nature of the Golgi impregnations on which I have had to depend to a large extent for tracing these intra-cranial fibers does not permit of demonstrating the connection between the nervus terminalis and the posterior portion of the sympathetic as clearly as would be the case with medullated fibers by the Weigert method, but the slightly diminished bundle of fibers of fig. 22 certainly continues rostrad along the carotid artery beneath the olfactory nerve, while the fibers of the nervus terminalis just as certainly become more or less distinctly separate from the olfactory nerve, after it enters the cranial cavity, and run near this same artery (figs, 10, 11, and 12).

It seemed quite probable to me at first that a connection might exist between the peripheral ganglion cells of the nervus terminalis in the nasal capsules of Amia and the post-optic sympathetic

system by way of the fifth nerve, since a sphenopalatine ganglion is mentioned in anatomical works in connection with the fifth nerve which sends fibers into the nasal capsule of higher vertebrates and Sheldon ('08) reported a ramus of the trigeminus nerve running into the nasal capsule of the carp. But in none of my Weigert preparations could I find fibers in Amia entering the nasal capsule in close proximity to the ganglion cells of the nervus terminalis. It may be that a few non-medullated fibers reach the ganglion cells here but no clear evidence of such a condition was found in searching my Golgi and Cajal preparations. The combined ophthalmic branches of the 5th and 7th nerves pass above the cavity of the nasal capsule in the membrane lining the inner side of the nasal bone (Allis,' 97, fig. 20), while the superior maxillary branch of the 5th and the buccalis branch of the 7th nerve send rami beneath the nasal capsule. It may be mentioned in this connection that no ganglion cells were found in Amia on the ophthalmic branch of the 5th nerve anterior to the point where it sends its ramus into the cranial cavity (fig. 25). Yet this may be different in other fishes and probably any branch found entering the cranial cavity of other fishes will be smaller than in Amia on account of the high degree of development of the meninges of the latter. It may be mentioned here that I have found nerve fibers along the blood vessels ventral of the forebrain of Ameiurus in Golgi preparations of young fishes, and that these preparations show evidence of essentially the same innervation of the pineal stalk as was found in Amia.

THE NERVUS TERMINALIS OF LEPIDOSTEUS AND TELEOSTS

As has already been mentioned, a ganglion was found on the olfactory nerve of Lepidosteus in a similar position to the one discovered by Allis in Amia. It can be readily recognized in specimens longer than 10 mm., and is located on the ventro-median side of the olfactory nerve. Stages of known age at close intervals have not been available for working out its early embryonic history. To give a detailed account of its later embryology would be to repeat much of what has been said of Amia. Con-

sequently, only a few points will be mentioned here. In a specimen about 12 mm. long cut sagitally, there was found a slender fibrous connection from the ganglion of about a dozen cells, running posteriorly beneath the olfactory nerve to the brain wall. As there was a small blood vessel on the brain wall just at this point, it cannot be said whether the fibrous connection is a root of the nervus terminalis or a fiber to the blood vessel. The cells are embryonic in appearance and many of them certainly do not possess nerve fibers until a much later date. As in Amia, the ganglion develops during very late embryonic stages. In a Lepidosteus over 85 mm. long, the ganglion of fifty cells or more, is located about half way from the olfactory bulbs to the nasal capsule; but in adults the main mass of cells lies peripherally in the nasal capsules. Nissl preparations of adult nasal capsules show ganglionic masses of these cells lying among the olfactory fibers at the base of the main folds of the Schneiderian membrane, of which there are about twelve. Also, the ganglion has been found in the young of what was thought to be the short-nosed gar (Lepidosteus platostomus).

While this manuscript was being written, preparations have been made which show conclusively that we have in teleosts a nerve very similar to the nervus terminalis of Amia and Lepidosteus. In the carp (Cyprinus carpio) there were found about three hundred ganglion cells scattered along a more or less distinct and separate strand of the olfactory nerve of a specimen about one-fourth meter long. In the historical sketch we have already cited Sheldon ('09) as having found in the carp the central connection of the nervus terminalis with the brain. The cells are somewhat larger than the sheath cells of the olfactory nerve, as in Amia, and are situated on the ventro-median side of the olfactory nerve in the nasal capsules. Their number diminishes as the olfactory bulbs are approached. The single Cajal preparation so far made to show the fibers, makes it evident that they are slightly coarser than the olfactory fibers, as in Amia, and that they are distributed everywhere in the nasal capsules, and that the main bundle turns dorsad from the ganglion cells to the region of the mid-rib. A full description of the condition in teleosts

will be reserved for another paper, but it may be said in this connection that with the help of Mr. T. S. Jackson, the development of a ganglionated nerve in two species of Ameiurus has been worked out in detail. The paper will soon be ready for publication and will show that there is the closest similarity in the development of the olfactory nerve and the nervus terminalis in Ameiurus when compared with the account given in this paper for Amia.

DISCUSSION AND THEORETICAL CONCLUSIONS

From the embryological history of the nervus terminalis given above for Amia, Lepidosteus, and the teleosts, it is clear that it is to be considered a component of the olfactory nerve rather than a separate segmental cranial nerve. This is in accord with the condition which Locy ('99) first described for Acanthias, but later ('05) he came to consider it as arising from a separate placode in the sharks. In the historical sketch I have cited the three points of similarity between the olfactory nerve and the nervus terminalis as pointed out by Pinkus ('05), and I may add here that the ganglion cells of the nervus terminalis have never been found farther caudad than the sheath cells of the olfactory nerve among which they arise in the fishes that I have studied.

We will next consider the homology of the nervus terminalis in the fishes. Something remains to be done embryologically on other fishes and there is need of bringing Locy's second account of its development in the shark into agreement with the work on other forms before the homology can be strengthened on the side of embryology, but its adult morphology shows that it is always distributed peripherally to the nasal capsules, that it is in close proximity to the olfactory nerve ventro-mesially and enters the forebrain not far from the neuropore. Also, it has generally been recognized by ganglion cells distributed along its course or more or less aggregated into ganglionic masses. It would be strange if such a nerve were not homologous throughout the fishes. We have quoted Locy ('03) and Pinkus ('05) as thinking so, and Sheldon ('09) is of the same opinion.

Herrick ('09) says the nerve which he found in the frog in a like position to the nervus terminalis in fishes is morphologically similar so far as our information extends. DeVries ('05) went farther and expressed himself as believing the nerve in fishes homologous with the nerve to Jacobson's organ (organon vomeronasale) in higher vertebrates and to be looked for everywhere in the vertebrate series. In reptiles Leydig ('97) found branching cells in the "inter-epithelial gland" at the base of the columnar epithelium of Jacobson's organ. Also in amphibians Rubaschkin ('03) reports nerve cells in the olfactory bulbs sending fibers peripherally into the nasal epithelium. We do not know what relation these fibers bear to the glandular structures sometimes found in the nasal capsule of amphibians.

Although Jacobson's organ is not well understood physiologically, it is pretty clear that it is morphologically a part of the nose. It develops as a cavity which evaginates from the nasal capsule, and in the adult of some macrosmatic mammals has been found by Miss Read ('08) to possess neurones similar to typical olfactory neurones. She found these to end centrally in glomeruli of the olfactory bulbs. Jacobson's organ has not been found as such in the fishes, but the olfactory nerve is quite generally divided into two rami peripherally, and this is true of some amphibians. It seems to me probable that the nerve to the organon vomeronasale is homologous with the median of the two rami in fishes rather than with the nervus terminalis, as DeVries has suggested. The nervus terminalis has generally been described as more intimately connected with the median of the two rami of the olfactoy nerve in fishes, and it may be that all, or a part only, of the nervus terminalis component is included in the nerve to Jacobson's organ in different species of higher vertebrates. In macrosmatic animals a large number of olfactory fibers remain, while in other cases the nerve to the organon vomeronasale may contain only the nervus terminalis component.

The condition of the nervus terminalis in sharks offers an apparent exception to the statement made above that the nervus terminalis of fishes is more intimately associated with the median ramus of the olfactory nerve. The nerve in sharks is median,

or ventro-median, of the olfactory nerve centrally, but takes a dorsal position peripherally and apparently is distributed mainly to the lateral half of the olfactory capsules. However, if the nasal capsules of the shark were rotated outward and upward into the position of the nasal capsules of most other fishes, it would have the same ventro-median position.

Details of anatomy of peripheral nerves are of most value when they are brought into relation with the function of the part of the body concerned. This has been the merit of the work of Sir Charles Bell, of Gaskell, and of the American neurologists on nerve components. We have already cited Pinkus as saying we are in ignorance of the function of the nervus terminalis, while Johnston has suggested its general cutaneous nature. We can readily see that if it is of somatic type, it is sensory rather than motor on account of the peripheral position of its ganglion cells. In my own experiments upon Amia there was no response detected in the nasal capsules when the olfactory nerve was stimulated near the olfactory bulbs with a strong faradic current. Neither did I detect changes in the rate of water flow through the nasal capsules nor blanching of the mucous membrane of the opened nasal capsules in these experiments. However, I do not think that the experiments prove that there is not vaso-motor control exercised by the nervus terminalis, since there was always much loss of blood in the operation of pithing, the parts are very small, and I have failed to get inhibition of the heart-beat when the vagus nerve was stimulated in the same way.

The fibers of the nervus terminalis probably do not belong to the same functional type as the fila olfactoria, for they differ in the type of their nerve cells, their location, time of development and central connections. No other specialized sense organ was found in the nasal capsules of the fishes studied and those described by Blaue ('84) in fishes and amphibians have been shown by subsequent authors to be collections of ordinary olfactory epithelium. As previously noted, Johnston has suggested that this nerve is somatic sensory of general cutaneous nature. In that event it might be thought to serve the tactile sense or some unspecialized sensibility similar to that which Parker ('08) or Sheldon

('09) have shown to be present in the skin of fishes. It is possible that it serves a visceral sensory function although the olfactory pit seems to develop as an invagination from the ectoderm.

The last suggestions receive but little support from the embryological development of the nervus terminalis. Both general cutaneous and some unspecialized visceral ganglia are developed from neural crest (Landacre, '08 and later unpublished observations), while the epibranchial and dorso-lateral placodes are thought to give rise mainly to ganglion cells of special sensory functions (gustatory and lateral line). The origin of the nervus terminalis from the olfactory placode, therefore, does not support the theory of its general cutaneous function. However, it should be pointed out in this connection that Beard ('88) described neural crest in the developing olfactory nerve and Johnston ('09) has just published an article in which he shows neural crest elements present in lower vertebrates in the region of the neuropore quite near the unpaired olfactory placode. The relation of neural crest with the olfactory placodes in this region has not been worked out fully in the earliest stages of Amia. It is possible that the unpaired olfactory placode (fig. 1) receives neural crest elements that enter the paired placodes to become the ganglion of the nervus terminalis later, but I was not able with my staining methods to differentiate the cells of the nervus terminalis until fully four days after the unpaired olfactory placode had disappeared. Yolk granules (fig. 1) obscure the details in very early stages of Amia and increase the difficulty of tracing possible neural crest elements through four days of embryological history.

Another possible interpretation of the nervus terminalis has already been referred to; viz: that its fibers are of sympathetic (visceral) type, probably vaso-motor. The embryological evidence here also is obscure for we know of no other case where sympathetic neurones are derived from ectodermal placodes. But if the paired olfactory placodes can be shown to receive neural crest cells, as suggested in the last paragraph, it offers a possible solution of the difficulty; for it is commonly thought that sympathetic neurones originate from neural crest (Jones, '05). We have mentioned that Carpenter ('06) found an apparent excep-

tion to the general rule that sympathetic ganglia are derived from neural crest. He found the cells that migrate along a motor nerve (the oculo-motor in the chick) giving rise to the sheath cells of that nerve and contributing neurones to the ciliary ganglion. The case is parallel with the condition found in the olfactory nerve and the nervus terminalis in Amia except that the migration of cells is from the olfactory placode rather than from the neural tube. Also, if it is granted that the cells in the pineal stalk in Amia are sympathetic, we have a case where the sympathetic cells originate from the neural tube direct without any apparent connection with neural crest in development.

We need further embryological and morphological data, as well as physiological evidence, in order to determine the function of the nervus terminalis. It may contain general cutaneous components along with sympathetic and possibly other elements in some forms of vertebrates. If it is largely vaso-motor, in the forms studied, as I have been led to think from the evidence, we may tentatively consider the fibers of the neurones entering the forebrain as preganglionic. The postganglionic neurones may be considered to be those that put the cells on the olfactory bulbs, and possibly some of those along the olfactory nerve intracranially into connection with the posterior part of the sympathetic on the one hand and with the cells in the nasal capsules on the other. We may summarize the evidence given in various places in the present paper that points to the sympathetic type of the nervus terminalis in Amia, as follows:

In addition to the point Allis made that the nervus terminalis develops at a time when the ciliary ganglion is developing, we have seen that its development is *pari passu* with the blood vessels which are always near it in the fishes I have examined. The same thing is true of other forms where the literature mentions the blood vessels. The cells in the periphery are many times more numerous than the fibers that were found entering the prosencephalon. These cells are multipolar in some instances and always aggregated into one or more ganglia or scattered like typical sympathetic ganglion cells. The same statement can be made of other forms mentioned in the literature. The fibers of

these nerve cells, peripherally as well as intra-cranially, were often found branching along the walls of the arteries. In other cases the nerve fibers arborize about the ganglion cells of the nervus terminalis, as sympathetic fibers are supposed to do. There is ample provision in Amia for connection with the post-optic sympathetic system, and it is difficult to account for a compact bundle along the arteries beneath the olfactory bulbs and the forebrain on any other supposition.

It is evident from the literature cited that the nervus terminalis cannot be considered a nerve peculiar to primitive vertebrates, as seemed probable so long as it was found in the generalized fishes exclusively. It appears more and more probable that there is a ganglionated nerve associated with the olfactory nerve throughout the vertebrate series. Aichel ('95) cites a number of authors who have found fibers in the nasal capsules differing from olfactory fibers. Some of these fibers are described as coarser than the olfactory fibers, while others are said to be smaller. In most instances they were attributed to the trigeminus nerve, but in light of our present knowledge the whole matter needs to be gone over again to determine whether they belong to the nervus terminalis, although we know that in some instances a ramus of the trigeminus nerve enters the nasal capsule.

SUMMARY OF RESULTS

1. This paper confirms the work of Allis in finding a ganglionated nerve in Amia, which is probably homologous with the nerve first discovered by Pinkus in Protopterus, and with the nervus terminalis found by Locy in a large number of sharks.

2. The ganglion of the nervus terminalis in Amia originates in common with the olfactory nerve, from an ectodermal placode.

3. In early stages the cells of the ganglion cannot be distinguished from the undifferentiated mass of cells which produce sheath cells of the fila olfactoria.

4. Incidentally, we have confirmed the results of recent investigators who find the olfactory neurones of the first order arising in the ectoderm and remaining there in the adult.

5. About one thousand nerve cells develop from the embryonic ganglion during late stages. Most of these lie in the nasal capsules in the adult but a few are found intra-cranially on the ventral side of the olfactory nerve in a more or less distinct ramus of this nerve.

6. The cells of the nervus terminalis lie in the dorsal sulcus between the two rami of the olfactory nerve in the adult nasal capsules. A few of them are scattered slightly dorsad of this position.

7. The nerve processes of the cells of the nervus terminalis in the adult possess neurofibrils and tigroid bodies are found in their cytoplasm, thus showing that they are functional nerve cells.

8. These cells show the three main forms characteristic of sympathetic ganglion cells.

9. Some of their nerve processes follow the arteries, while others arborize about other cell bodies of the ganglion of the nervus terminalis.

10. Not more than about forty axones of the thousand ganglion cells of the adult were found joining the olfactory bulbs. Many of these fibers pass through the bulbs to end in the prosencephalon proper.

11. Ganglion cells of the same general character were found increasing slightly in numbers as the nervus terminalis approaches the olfactory bulbs.

12. We have confirmed Allis in finding fibers that seem to belong to the nervus terminalis continuing caudad ventrally of the olfactory bulbs to the region of the optic chiasm. These fibers were not found to enter the brain, as Allis seems to have suspected they would do, probably on account of the condition described for Protopterus; but connect with the post-optic sympathetic system by way of an intra-cranial sympathetic system.

13. Golgi and Cajal preparations show that the blood vessels everywhere within the cranial cavity are innervated. Also, the paraphysis is innervated.

14. A ramus of the trigeminus nerve, probably derived from the profundis nerve, enters the cranial cavity dorsally opposite the anterior commissure. Some of these fibers are medullated and supply the paraphysis tubes.

15. Ganglion cells are found along this ramus of the trigeminus nerve intra-cranially. Their maximum number is about thirty on a side in the adult.

16. There is reason to think the paraphysis has its intrinsic nerve supply.

17. The epiphysis, or pineal stalk, is innervated richly with a type of cells and fibers much like the sympathetic plexus found in the intestinal walls of vertebrates. It sends about forty fibers centrally into the brain past a glandular structure at its base. Some of these fibers seem to pass to the habenulæ, but the great majority were lost in proximity to the walls of the third ventricle.

18. There is some evidence that the innervation of the pineal stalk, also, is connected with the post-optic sympathetic system through the trigeminus nerve.

19. In a number of specimens there was found to be a bundle of about a dozen fibers running in the fold of pallium between the halves of the forebrain in adults. This is capable of connecting the nervus terminalis with the post-optic sympathetic system, but the main connection is probably by a bundle ventrad from the entrance of the trigeminus nerve into the cranial cavity and thence along the internal carotid artery to the olfactory bulbs.

20. The nervus terminalis has been found in Lepidosteus and in teleosts.

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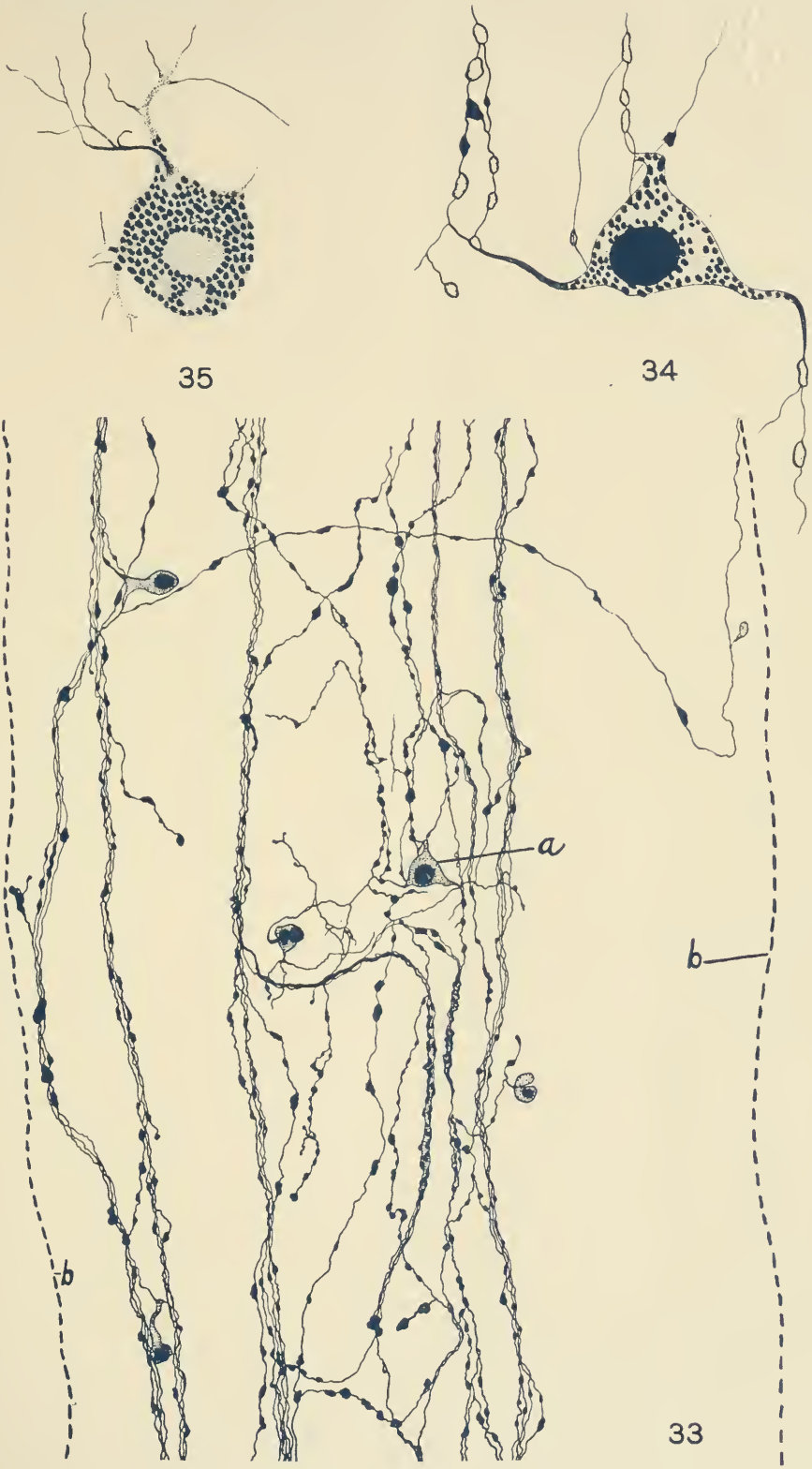
Description of Figures

Fig. 33. Intra-vitam methylen blue impregnation of the nerves of the pineal stalk (epiphysis) of adult Amia, as seen from the surface of a total mount showing the relation of the cells and their processes to the longitudinal fibers. Lateral limits of pineal stalk at *b*. × 408.

Fig. 34. From the same preparation as previous figure to show details of a cell at point *a*. Note the Nissl bodies and the similar structure of the different cell processes. × 1500.

Fig. 35. From the same preparation as the two previous figures, near the edge of the mount where there were not so many overlying layers of the meninges to obscure the details. × 1500.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CORRECTIONS

Page 122, line 23
For "15 to 16," read "17 to 18"

Page 138, line 5
For "P. 19" read "P. 134"

Page 143, next to bottom line
For "constituents the" read "constituents of the"

ON THE PERCENTAGE OF WATER IN THE BRAIN AND IN THE SPINAL CORD OF THE ALBINO RAT

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The Wistar Institute of Anatomy and Biology

WITH FIVE FIGURES

The object of this study has been to obtain a continuous record of the change in the percentage of water in the central nervous system of the albino rat during its life cycle, and to correlate this with the other important changes in the nervous system which are commonly recognized. These results in turn should put us in a position to determine to what extent and in what way this character may be modified.

Although it has long been known that at birth the percentage of water in the central nervous system was much greater than at maturity, yet the change in this character through the life cycle has never been systematically followed, and it thus happens that there are no other extensive records with which to make comparison. The relations of existing data to this investigation will be discussed later on.

The data used for the following study were largely obtained from the same animals which furnished the records employed for the two previous researches on the weight of the brain and of the spinal cord of the albino rat under different conditions of age, body-weight and body-length (Donaldson '08 and '09) although many cases have been necessarily excluded because the percentage of water had not been determined. On the other hand, a few new records have been added to the original series.

In carrying on this work, which has extended through a number of years, I have been greatly assisted by Dr. Hatai, as well as by two of my former students, Dr. Polkey and Dr. Whitelaw, both of whom made a number of the determinations of water under my

directions, and to all of these gentlemen I wish here to express my obligations for assistance.

Technique. The determination of water has been made for the entire encephalon severed from the cord at the level of the first spinal nerve, and for the entire cord, the spinal nerves having been clipped away at their origin from the cord. The rats used were chloroformed, eviscerated and rapidly dissected. No special device for preventing evaporation during dissection was used.

The percentage of water applies therefore to the nerve structures proper, surrounded by the meninges and containing such blood as usually remains after the foregoing treatment.

The details of the technique according to which the brain and spinal cord were removed have been already given (see Donaldson '08, p. 346). Each brain or cord was placed in a small glass-stoppered weighing bottle, and after being weighed in the fresh state, was dried in a closed water bath which had a temperature ranging from 85°–95° C. and then was cooled in a dessicator over sulphuric acid, and reweighed.

The brain took somewhat longer to dry as a rule than the spinal cord, but usually seven days in the water bath served to bring it to a constant weight. At various times objections have been raised to the determination of the percentage of water by the use of heat. The other method which is most approved is that of drying the material at the room temperature or somewhat above, in a vacuum over sulphuric acid.

A comparison of these two methods has been made for the brain and cord of the rat, but no significant differences have thus far been found. I shall, however, reserve the discussion of the data on which this statement is based for another occasion.

The percentage of water in the brains of albino rats of different body weights. The number of cases is 409 males and 212 females. The mean values for the percentage of water in the brain for given body weights differing by 10 grams, as determined by a correlation table, are entered in table 1.

The examination of table 1 shows for the brain a relative loss of water amounting to about ten units between birth (body weight 5 grams) and the end of the series. This loss is most

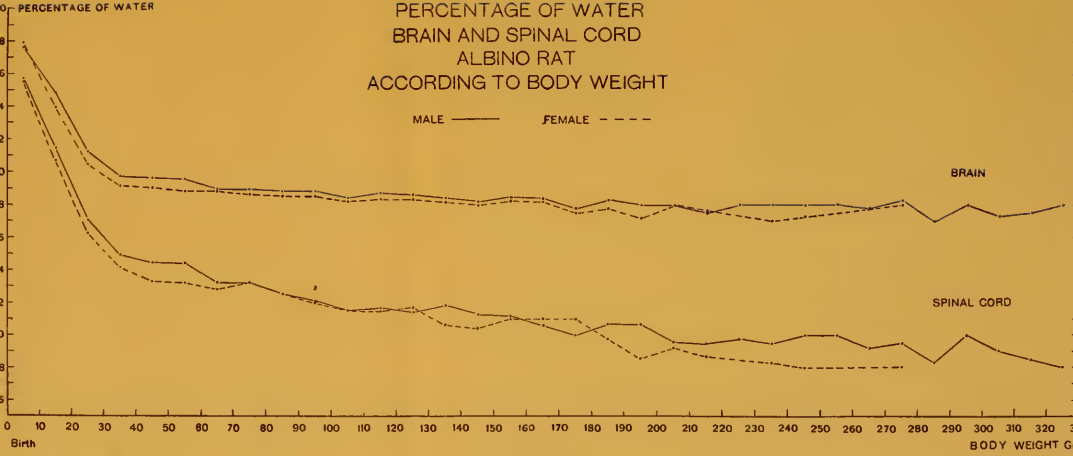

CHART 1

To show the changes in the percentage of water in the brain and in the spinal cord of the albino rat of different body weights. The data for the two sexes are plotted separately.

TABLE I.

The mean values of the percentage of water in the brain and spinal cord of the albino rat.[1] *Both sexes arranged according to body weights, increasing by 10 gram increments.*

BODY WEIGHT	PERCENTAGE OF WATER			
	BRAIN		SPINAL CORD	
	Male	Female	Male	Female
Grams.				
5	87.6	87.9	85.7	85.5
15	84.8	83.9	81.4	80.7
25	81.2	80.4	77.0	76.2
35	79.7	79.1	74.9	74.1
45	79.6	79.0	74.4	73.3
55	79.5	78.8	74.4	73.2
65	78.9	78.8	73.2	72.8
75	78.9	78.6	73.2	73.2
85	78.8	78.5	72.5	72.5
95	78.8	78.5	72.1	72.0
105	78.4	78.2	71.5	71.5
115	78.7	78.3	71.7	71.5
125	78.6	78.3	71.4	71.7
135	78.4	78.2	71.8	70.6
145	78.2	78.0	71.3	70.4
155	78.5	78.3	71.2	71.0
165	78.4	78.2	70.6	71.0
175	77.8	77.5	70.0	71.0
185	78.3	77.8	70.7	69.8
195	78.0	77.2	70.7	68.6
205	78.0	78.0	69.6	69.3
215	77.5	77.7	69.5	68.7
225	78.0		69.8	
235	78.0	77.0	69.5	68.3
245	78.0	77.3	70.0	68.0
255	78.0		70.0	
265	77.8		69.2	
275	78.3	78.0	69.5	68.0
285	77.0		68.3	
295	78.0		70.0	
305	77.3		69.0	
315	77.5		68.5	
325	78.0		68.0	

[1] For reasons similar to those previously given (see Donaldson '08, pp. 156–157), the individual records are not printed. These however are on file and copies of them may be had by application to the Director of the Wistar Institute.

rapid at the time when the brain is growing most actively. Table 1 further shows that the percentages for the females are in general slightly less than those for the males of the same body weights. Chart 1, which is based on table 1, exhibits this relation.

As we shall see later, the percentage of water in the central nervous system is more closely correlated with age than with the body weight or brain weight. Nevertheless, it will most often occur that it is desired to estimate the probable percentage of water in cases where the weight of the body or brain alone are known, and the foregoing table 1 furnishes the means of doing this for animals which have been grown under the ordinary normal conditions.

It has been already demonstrated (Donaldson '06) that for a given age, the body weight of the female is less than that of the male, consequently the comparison in each case is here between males that are younger than the females with which they are contrasted, and as increasing age is an important factor tending to reduce the percentage of water, it follows that the males, which are younger, should show, as they do, a slightly greater percentage of water.

Percentage of water in the spinal cord. In the spinal cord the relative loss of water with increasing body weight is greater than in the brain, being from 15 to 16 units. Although the initial percentage is somewhat less, yet the subsequent loss is regularly more rapid than that in the brain. The percentage of water in the two sexes is related in the same way as in the brain. The observations are given in table 1 and in chart 1.

Percentage of water in relation to age. To support the suggestion that the males in the foregoing tables show a greater percentage of water, because they are younger than the females, the data have been rearranged according to age. In many cases the age was not known, and this reduces the number of records to 358 males and 169 females. The results in the form of mean values, based on a correlation table are given in table 2 and plotted in chart 2, the entries being made for ten day intervals. When thus arranged, it appears that in the brains of males and females of like age, the percentage of water is similar.

For the brain, the records show in both sexes ranges in the percentage of water in the different age groups as follows:–

AGE			PERCENTAGES
0–10 days	total range	3 units	86–89
10–20 days	total range	5 units	82–87

From 20 to 100 days the range diminishes, and after this latter age it does not amount to more than one unit. The ranges for the spinal cord are less than those for the brain.

It will naturally be asked whether among individuals belonging to the same litter, reared under similar conditions and killed at exactly the same age there is any difference in the percentage of water between those having relatively heavy brains and spinal cords and those in which these organs are relatively light. This question seems to be answered in the negative by the result of 25 pairs of observations recently made.

The figures are as follows:—

	PER CENT OF WATER	PER CENT OF WATER	
Heavy brains	78.651	72.436	Heavy cords
Light brains	78.649	72.465	Light cords

In both instances as is seen, the differences found are too small to be significant. It may be added that the weight of the light brains was about 96 per cent that of the heavy, and similarly the weight of the corresponding light spinal cords about 93 per cent. Such differences as we find therefore among specimens of the same age must depend on some other cause than the individual variations in the weight of the central nervous system.

I feel sure that the irregularities seen in the curve for the cord, chart 2, 95–115 days, are purely incidental and would not appear on repeating the observations.

At the same time it is seen that the percentage of water in the female spinal cord after the period of rapid growth, is in general a trifle higher than in the male. This is an unexpected result. The mean difference as determined from those entries in table 2 where there are data for both sexes at a given age (i. e., up to 230–240 days) is 0.36 per cent. At the moment this difference is most readily explained as one effect of the passive lengthening

TABLE 2

The mean values of the percentage of water in the brain and spinal cord of the albino rat. Both sexes arranged according to age, increasing by 10 day increments.[2]

AGE IN DAYS	PERCENTAGE OF WATER			
	BRAIN		SPINAL CORD	
	Male	Female	Male	Female
0–10	87.4	87.4	84.8	84.8
10–20	83.7	83.4	80.5	80.3
20–30	81.3	81.6	77.2	77.1
30–40	79.4	80.0	74.3	74.8
40–50	79.2	79.0	73.9	73.7
50–60	79.0	79.3	72.9	74.2
60–70	79.3	78.8	74.5	73.2
70–80	78.9	78.8	72.9	73.8
80–90	78.3	78.8	72.8	73.8
90–100	78.7	79.0	73.0	74.1
100–110	78.3	78.0	70.0	70.8
110–120	78.6	78.7	71.3	72.5
120–130	78.3	78.2	71.6	71.7
130–140	78.2	78.0	70.0	71.0
140–150		78.0		72.0
150–160	78.1	78.0	70.6	70.8
160–170	78.2	78.3	71.0	71.3
170–180		78.0		71.0
180–190	78.0	79.0	71.0	71.5
190–200				
200–210	78.0	79.0	71.0	72.0
210–220	78.3	78.3	71.0	71.7
220–230	78.7	78.3	72.2	71.0
230–240	78.5	78.0	71.0	71.0
240–250				
250–260				
260–270				
270–280				
280–290				
290–300	78.5		72.0	
300–310		77.4		68.2
310–320		77.3		68.0

[2] Note that the values here given begin with 0–10 days, i..e, a mean age of *five days after birth.* Hence the initial percentages are less than those in table 1 which gives the values at 5 grams, approximately the weight at birth.

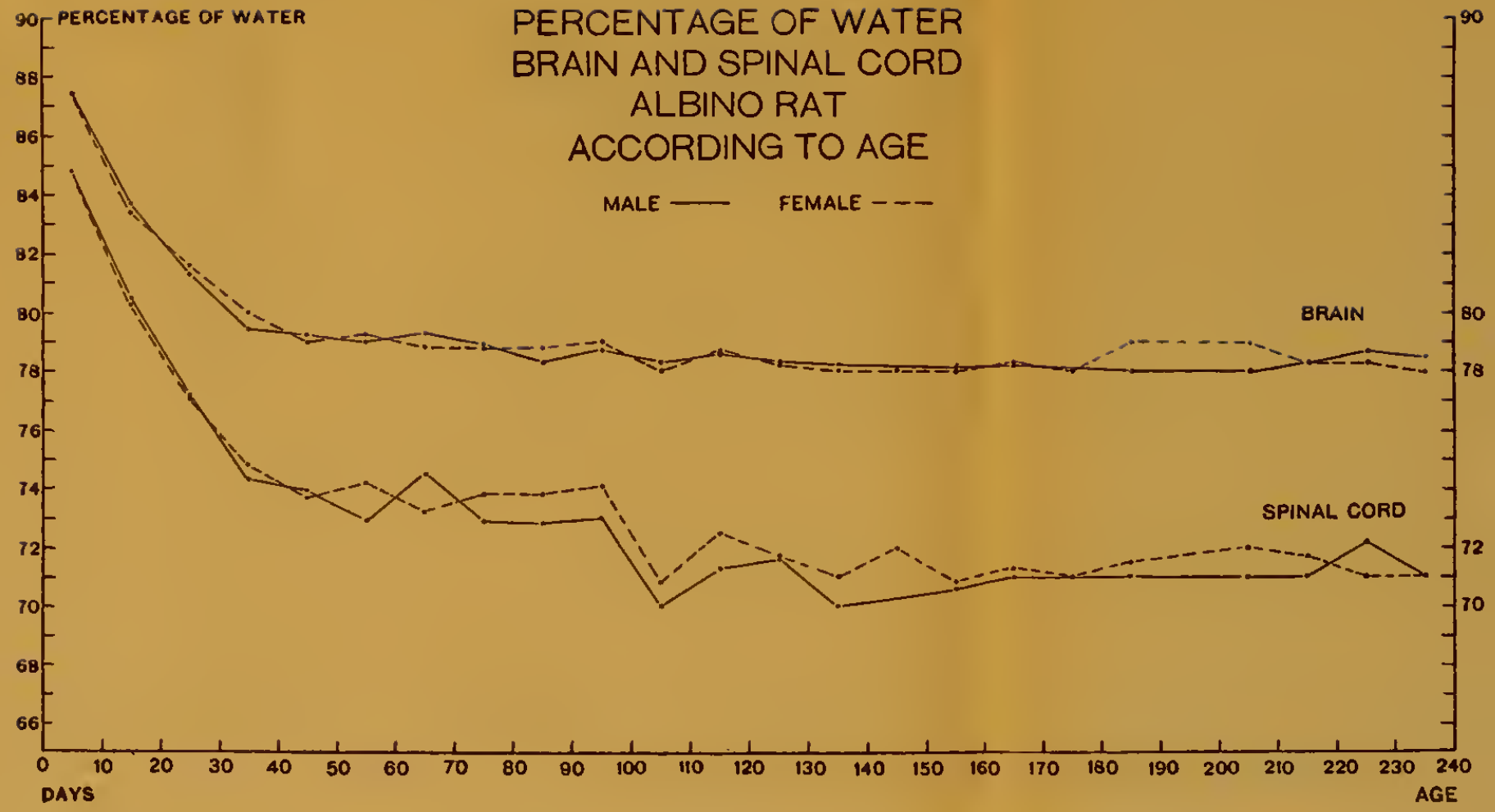

CHART 2

To show the changes in the percentage of water in the brain and in the spinal cord of the albino rat at different ages. The data for the two sexes are plotted separately. It is to be noted that the first entry is at the mean age of five days.

of the spinal cord which for a given age is relatively somewhat greater in the male than in the female (Donaldson, '09 pp. 163–164). The effect of this lengthening would be to diminish the percentage of water. The influence of passive lengthening is discussed more fully later on.

Theoretic curves. When we take the more extensive series of mean values which is that for the males as given in table 1, and draw the theoretic curves based on them, we obtain the relations shown in chart 3, the entries being arranged according to body weight. The data for this chart are given in table 3. For the formulas for these curves, I am indebted to Dr. Hatai.

The formulas for the percentage of water in the brain of the male albino rat are as follows:

Up to a body weight of 30 grams

$$y = 99.5 - 12.6 \log (x + 3.5) \quad [1]$$

and from a body weight of 30 grams on

$$y = 82.62 - 2 \log (x - 10) \quad [2]$$

In the case of the percentage of water in the spinal cord of the male albino rat we have for body weights up to 35 grams

$$y = 94.9 - 12.8 \log (x) \quad [3]$$

and from a body weight of 35 grams on

$$y = 85.2 - 6.5 \log (x) \quad [4]$$

In all these formulas y = the percentage of water and x = the weight of the body in grams.

The formulas are of the same type as those used to express the growth changes described in several previous investigations (Donaldson '08, '09; Hatai '09), and have their main value as convenient expressions of the several series of observations.

Calculations (based on table 1, down to and including the entries for 275 grams body weight) show that in general for given body weight, the females which are under these conditions relatively older as compared with the males, have a percentage of water lower by 0.37 per cent in the brain and 0.60 per cent in the spinal cord. The theoretic values for the female can therefore be obtained approximately by applying these corrections to the determinations here given for the males.

Having thus presented the data on the percentage of water

TABLE 3

Giving the values of the percentage of water in the brain and spinal cord of the male albino rat, calculated according to the formulas given above. Brain: formulas 1 and 2; spinal cord: formulas 3 and 4. For comparison the observed values for the male, taken from table 1, are repeated here. Arranged according to body weights

BODY WEIGHT	PERCENTAGE OF WATER			
	BRAIN MALE		SPINAL CORD MALE	
	Calculated	Observed	Calculated	Observed
grams				
5	87.79	87.6	85.96	85.7
10	85.26		82.10	
15	83.50	84.8	79.80	81 4
20	82.24		78.26	
25	81.17	81.2	76.98	77.0
30	80.22		75.96	
35	79.83	79.7	75.16	74.9
45	79.53	79.6	74.45	74.4
55	79.31	79.5	73.89	74.4
65	79.14	78.9	73.42	73.2
75	78.99	78.9	73.01	73.2
85	78.87	78.8	72.66	72.5
95	78.76	78.8	72.34	72.1
105	78.66	78.4	72.06	71.5
115	78.58	78.7	71.81	71.7
125	78.50	78.6	71.57	71.4
135	78.43	78.4	71.35	71.8
145	78.36	78.2	71.15	71.3
155	78.30	78.5	70.96	71.2
165	78.24	78.4	70.79	70.6
175	78.19	77.8	70.62	70.0
185	78.13	78.3	70.46	70.7
195	78.09	78.0	70.31	70.7
205	78.04	78.0	70.17	69.6
215	78.00	77.5	70.04	69.5
225	77.96	78.0	69.91	69.8
235	77.92	78.0	69.79	69.5
245	77.88	78 0	69.67	70.0
255	77.84	78.0	69.56	70.0
265	77.81	77.8	69.45	69.2
275	77.77	78.3	69.34	69.5
285	77.74	77.0	69.24	68.3
295	77.71	78.0	69.15	70.0
305	77.68	77.3	69.05	69.0
315	77.65	77.5	68.96	68.5
325	77.62	78.0	68.87	68.0
470	77.30		67.80	

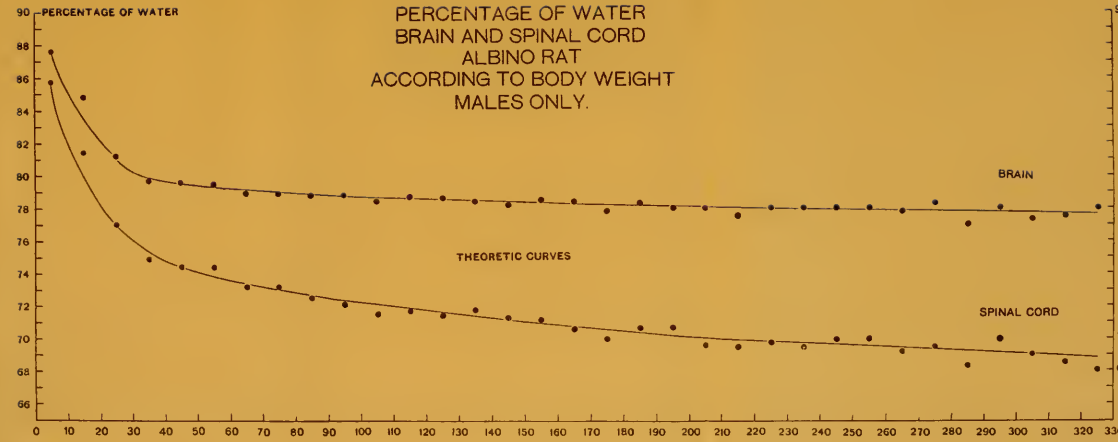

CHART 3

Theoretic curves showing the changes in the percentage of water in the brain and in the spinal cord of the male albino rat at different body weights. The dots ● show the observed mean values.

in the brain and cord of rats according to age and to the normal body weight, we pass to the question of the extent to which the percentage of water may be modified experimentally under special conditions. The amount of modification which has been experimentally induced is thus far extremely slight, nevertheless some deviation seems to occur. The evidence is as follows:

(*a*) *Some conditions which increase the percentage of water in the brain and cord.* Dr. Watson ('05) when working on the effects of the bearing of young on the weight of the central nervous system in the albino rat, noted that the mated animals had both heavier brains and heavier cords. He noted also that the mated rats, as compared with the unmated of like age, had the following percentages of water:

	NO. OF CASES	PERCENTAGE OF WATER	
		Brain	Cord
Female mated	(8)	77.47	68.51
Female unmated	(10)	77.37	68.29

This shows that the mated rats had in the brain 0.10 per cent more water than the unmated, and in the spinal cord 0.22 per cent more. Thus, even though the brain and cord in the mated series were absolutely heavier, yet if the higher percentage of water be taken as an index of a lesser maturity, the central nervous system of the mated rats must be regarded as physiologically younger. Such slight differences would, of course, not be worthy of remark if they had been obtained merely by the averaging of widely varying data, but in this case comparisons were made by Watson in five different groups for the brain, and five for the cord, and in only one (in the cord) out of the ten comparisons, did the mated rats show a smaller percentage of water. Thus though the difference is small, it was found to occur in the same sense in nine cases out of the ten. This seems to justify the conclusion which Watson drew that mated female rats had a slightly higher percentage of water in the brain and spinal cord than the unmated females belonging to the same litters.

Hatai ('07) also has made observations on the modifications of body growth as the result of which the percentage of water in the central nervous system was slightly increased.

When young rats were underfed for three weeks and then returned to a normal diet, Hatai found that their subsequent increase in body weight was somewhat more rapid than that of the control group, and in the case of the males, the final weight even greater. Hatai's table IV ('07) is here repeated.

TABLE 4.

	ENCEPHALON PER CENT	SPINAL CORD PER CENT
Male controls	77.50	69.71
Male experimented	77.75	70.05
Female controls	77.50	69.40
Female experimented	77.75	70.10

Taking both sexes together, the experimented groups, as shown in the above table 4, had on the average a percentage of water in the brain greater by 0.25 per cent and in the cord by 0.52 per cent. As will be observed, this treatment produced a rather greater alteration in the percentage of water than was obtained by Watson in the case of the mated and unmated females.

In the foregoing instance there were fourteen pairs of brains between which comparisons were made, and in thirteen of these the experimented rats show a greater percentage of water. In the case of the spinal cord, eleven pairs out of a total of fourteen show the experimented rats to have the greater percentage of water, so that here again although the variation induced by the treatment is not great, yet a slight change seems to be really effected.

In another series of observations Hatai ('08) got still more marked differences in the percentage of water. In this case there were seven pairs of contrasted individuals. Seven individuals were used as controls and seven others, from the same litter, fed with small quantities of a varied diet and thus stunted. When these latter had attained an average age of about 140 days, they were put on a full normal diet for thirty days and then both lots were killed and examined at the same time.

During the thirty days of normal feeding, the stunted rats grew in weight and length. When killed at this time it was found that the stunted rats had in both brain and cord a distinctly

greater percentage of water than did the controls. The difference is in the same sense in all the pairs and for both the brain and spinal cord. The average figures are as follows.—

AVERAGE AGE	NO. OF CASES	PERCENTAGE OF WATER	
		Brain	Cord
170 days	7 stunted	78.618	72.613
170 days	7 controls	78.378	71.076

As the figures show, the percentage of water in the stunted group is greater by 0.24 per cent in the brain and 1.53 per cent in the cord.

The difference in the case of the brain is about that found in the preceding investigation, but that in the cord is much greater. The reason for the greater percentage of water in the case of the spinal cord requires still to be investigated.

The foregoing conditions are the only ones which at the moment have been shown to increase the percentage of water in the central nervous system of the rat, and in all cases this increase seems to be associated with more vigorous growth processes.

(*b*) *Some conditions which decrease the percentage of water in the brain and spinal cord.* On the other hand, in 1904 Hatai determined that in rats killed at the end of three weeks of underfeeding the experimented rats, though initially heavier, had on the average only about 44 per cent of the body weight of the controls.

This result was due not only to an arrest of growth, but to an actual loss, as measured by changes in the weight of the entire body and also of the brain. At the termination of the experiment, the brain weight in the underfed group was about 11 per cent less than in the controls, approximately two thirds of this deficiency being due to the arrest of growth, and one third (4.3 per cent) to actual loss (see table IV, Hatai '04). On the other hand, the percentage of water in the brain was

79.11 per cent in the controls
78.91 per cent in the experimented,

thus showing a deficiency of 0.2 per cent in the latter. If the process of the *reduction* of the percentage of water had been

stopped by the underfeeding, which stops the growth as indicated by the body weight and the brain weight, we should have found the higher percentage in the experimented rats.

As further evidence that the disturbance of the growth process involves but slightly the changes in the percentage of water correlated with increasing age, we have the data in this same paper by Hatai given in Table IV, series II where the control group was killed and examined at the beginning of the experiment. Here the percentages are

79.01 for the control rats
78.71 for the experimented rats

giving a difference of 0.3 per cent.

The difference in this case is greater than in the preceding because not only is the percentage of water in the experimented group slightly diminished by the treatment, but also because the experimented group was three weeks older than the controls at the time of killing, thus giving a total loss of 0.3 per cent in series II against 0.2 per cent in series I, where both controls and experimented rats were killed at the same time. This again supports the view that underfeeding does not arrest the changes in the percentage of water characteristic for advancing age, but may rather hasten them.

The weight of water in the brain and spinal cord. The preceding descriptions have been given in the terms of the percentage of water. A better view of the changes taking place can be obtained however by following the suggestion of my colleague, Dr. Hatai, and showing the changes in the absolute weight of the water in the brain and cord at different weights of these parts. This eliminates the time factor which has modified the previous forms of presentation, and gives a simple and suggestive picture of the changing relations between the water and the solids.

The determinations thus made are given in table 5 and have been also plotted in charts 4 and 5

The following table 5 shows that for the successive increments of weight, the female brain has less water than the male brain of like weight. This is undoubtedly due to the fact that under

the conditions of comparison, the female brains are the older. Owing however to the relatively large interval of brain weight used in the correlation tables, from which the means in table 5 were obtained, the absolute weights for the amounts of water increase irregularly, and this in turn makes the progressive per-

TABLE 5

The weight of water present in the brain and in the spinal cord according to the absolute weight of these organs. Sexes distinguished. Based on the entire series of records for both sexes. Mean values determined from correlation tables.

BRAIN WEIGHT	AMOUNT OF WATER — BRAIN		SP. CD. WEIGHT	AMOUNT OF WATER — SPINAL CORD	
	Male	Female		Male	Female
grams	*grams*	*grams*	*grams*	*grams*	*grams*
0.25	0.208*	0.175*	0.03	0.028	0.027
0.35	0.325	0.290	0.07	0.067	0.062
0.45	0.350	0.400	0.11	0.085	0.083
0.55	0.510	0.450	0.15	0.116	0.110
0.65	0.600		0.19	0.147	0.146
0.75	0.650	0.650	0.23	0.176	0.183
0.85	0.736	0.700	0.27	0.199	0.191
0.95	0.817	0.800	0.31	0.230	0.226
1.05	0.860	0.850	0.35	0.250	0.248
1.15	0.950	0.938	0.39	0.280	0.275
1.25	1.025	1.012	0.43	0.308	0.308
1.35	1.088	1.067	0.47	0.340	0.318
1.45	1.150	1.143	0.51	0.354	0.358
1.55	1.234	1.232	0.55	0.390	0.390
1.65	1.304	1.294	0.59	0.412	0.398
1.75	1.359	1.355	0.63	0.434	0.430
1.85	1.450	1.444	0.67	0.465	0.450
1.95	1.530	1.520	0.71	0.473	0.470
2.05	1.636	1.550	0.75	0.520	
2.15	1.650				

*Not plotted.

centages still more irregular. However, a second series of calculations based on the theoretic curve for the percentage of water (see table 3 and chart 3) agree so well with the observed results here given that the general correctness of the latter may be accepted.

The significance of table 5 is made more evident by plotting the data on a base line giving either the weight of the brain or of the spinal cord. It is then seen that the records for the weight of water lie in an approximately straight line.

Weight of water in the brain. Beginning with the brain, chart 4, it is seen that when the lines representing the actual weight of water are contrasted with the dotted line, showing the amount of water necessary to maintain the percentage constant at its initial value, the former ascend less rapidly. Further inspection shows that the lines representing the increments of water as observed are slightly convex. This is true for both sexes. We will first consider in detail the relations as thus shown for the males.

A straight line drawn between the terminals for the male curve corresponds to an average of 73.6 per cent of water in the increments of weight after a brain weight of 0.35 grams. Since, however, the curve is slightly convex, it is better represented by two straight lines, one drawn from the initial entry to the entry above the brain weight of 1.05 grams, and the second from this latter to the final entry at 2.15 grams. The angle of the former line corresponds to 76.4 per cent. of water and that of the latter to 71.8 per cent.

From this it appears that the earlier increments of brain weight have a somewhat greater percentage of water than those acquired later.

It is to be noted however that the earlier period comes to an end when the animal weighs only 17 grams, and is about 15 days old (see chart 4) although by this age the very rapid growth of the brain in weight has been completed. (See Donaldson '08, plate III, chart 3.)

With slight differences, which are not significant, the relations here described for the males hold for the females also, but it is hardly necessary to give the determinations in detail.

Such are the general relations of the increase in the weight of water with the increase in brain weight. By these relations several facts are shown.

First. The proportion of water in the brain diminishes with

increasing brain weight; a fact already demonstrated by the previous tables and charts.

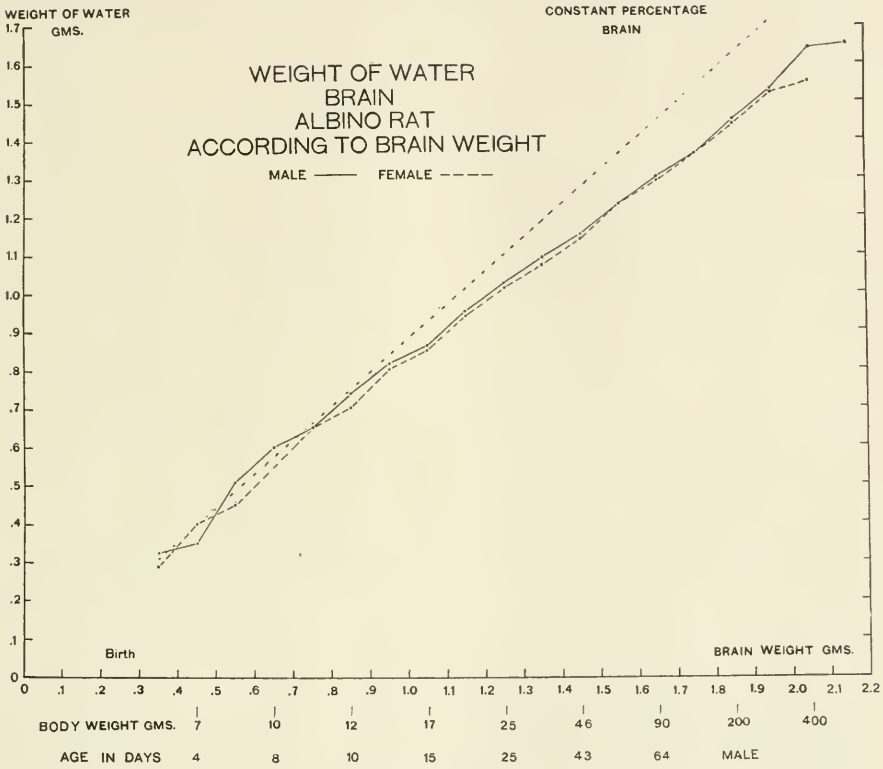

CHART 4

To show the absolute increase in the weight of water corresponding to the increase in brain weight. The data for the two sexes plotted separately. The first entry is for a brain weight of 0.35 grams. Below are given the body weights and the ages in days for the several brain weights. The dotted line indicates the amount of water which would be required to maintain the percentage at the initial value.

Second. The increments of brain weight are characterized by a continuous though small diminution in the percentage of water in the successive increments, the more rapid diminution occurring after the first fifteen days of life.

Third. After the rat has attained about fifteen days of age, the percentage of water in the increment of weight becomes

approximately constant for the remainder of the life cycle, having an average value of 71.8 per cent. This value forms a limit towards which the percentage of water in the entire brain slowly falls.

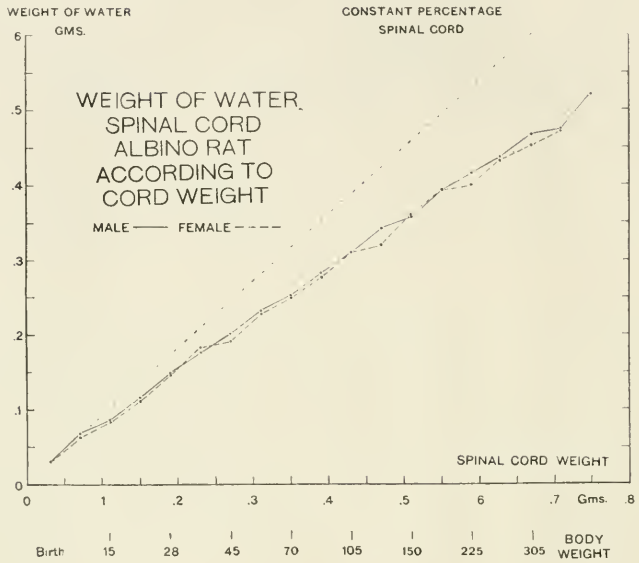

Chart 5

To show the absolute increase in the weight of water corresponding to the increase in the weight of the spinal cord. The data for the two sexes are plotted separately. Below are given the body weights for the corresponding spinal cord weights. The dotted line indicates the amount of water which would be required to maintain the percentage at the initial value.

Fourth. During the period of most active medullation, i. e., from 20–100 days, the percentage of water in the increments of brain weight does not indicate that the medullary sheaths which are being rapidly formed, possess a percentage of water less than that of the axones on which they appear.

It follows from the foregoing that as the amount of water in the brain at any time after birth is the sum of the amount present at birth (a constant) plus the successive increments, the percentage of water will diminish most rapidly at first. As the brain becomes heavier, and the increments form a greater proportion of the total

weight, the rate at which the percentage of water diminishes will become slower and slower. At first glance it may be difficult to harmonize these data on the absolute weight of water with the rapid fall in the percentage of water as it appears in charts 1 and 2 based on body weight and on age. If, however, the precocious growth of the brain and spinal cord is recalled, a reference to chart 4 in which the body weight and the ages are given below the brain weights, will serve to make the matter clear.

Weight of water in the spinal cord. The foregoing relations as described for the brain hold true for the spinal cord of both sexes as well, with the difference that in the cord the percentage of water in the total increment from the first to the final entry is less than in the brain, being 68.3 per cent. The percentage of water in the increment during the first 15 days of life is on the average 70.4 and after that 67.9. The record in the case of the cord therefore is more nearly represented by a single straight line than in the case of the brain, but like conclusions can be drawn from the study of the data on the spinal cord as here presented.

Explanation of the change. It still remains to attempt an explanation of the course followed by the percentage of water through the life cycle, and also to explain why even at birth the brain has more water than the cord, as well as why it shows a smaller fall in this percentage during the life cycle.

In the interests of such a general explanation, let us consider first the condition of the brain and the cord at birth.

In the albino rat at birth, both brain and spinal cord are unmedullated, and both are very watery. Both are composed of gray matter in the strict sense, and growing axones, also gray in color. The nerve elements are enmeshed in supporting tissues and vessels.

In the brain the probability is that the supporting tissues, as well as the vessels, form a slightly smaller fraction of the total mass than in the cord. Cell division in the brain is continued longer after birth than in the cord, while medullation in the brain begins later than in the cord, and is less rapid. During subsequent growth, medullation is most actively carried on from the age of about twenty to one hundred days.

Between birth and maturity the proportional increase in the

weight of the brain is only about two fifths that of the spinal cord (Donaldson '08, p. 355 and p. 358) and at maturity the relative amount of white matter in the brain is much less than in the spinal cord (Donaldson and Davis '03; Watson '03). Such are the characteristics of these two portions of the central nervous system which are of interest to us in connection with the percentage of water.

Explanation of the greater percentage of water in the brain at birth. The greater percentage of water in the brain at birth may be connected with some of the facts just enumerated, namely, the lesser maturity of the brain, as indicated by the longer continuance of cell division, by the later onset of medullation, and by the lesser proportion of supporting tissues and other non-nervous constituents. All of these conditions would tend to give the brain a higher percentage of water.

During the subsequent growth, the slower diminution of the percentage of water in the brain is due to the fact that the relative increment of water is greater than in the case of the cord (see charts 4 and 5). This however is again no explanation and leaves the conditions which control the increment of water in each division of the system still to be described.

As can be seen from inspecting chart 4 it is possible to express the events taking place by assuming that the initial weight of material in the brain maintains its initial percentage of water and that each of the subsequent increments in weight from just after birth to old age has a relatively low and slightly diminishing percentage of water. Such a statement however is purely formal.

What probably takes place is this: Starting with a given percentage of water in the brain or cord, this percentage continually diminishes as the formed material becomes older—at the moment of formation, however, the young material subsequently added most probably has a relatively high percentage of water, and the percentage we obtain at any given age is therefore the mean of these several values. The rate at which the percentage is falling off at any moment, together with the general slowing of the growth process—requiring a longer and longer time to add the same increment of weight to the brain the older the brain be-

comes—is so adjusted as to yield after the period of more rapid growth of the brain, the rather simple relations of a nearly constant weight of water for the same increment of total weight.

In this connection the analysis of the brain and cord should however be carried one step further. Both are composed of gray matter (substantia grisea) and axones, plus the supporting elements, the axones being more or less medullated according to locality and age. In the case of the rat, it has not been possible to study the changes in the percentage of water in the gray matter alone. We know however from a number of studies on man—on the cortical gray and the gray of the corpus striatum—that the change in the percentage of water in the substantia grisea with age, is much less—less than one-half—that in the axones (white matter). This has a bearing on the percentage of water in the brain as contrasted with the cord, because the brain has relatively less axone substance in it. Moreover the maturing of this substance is slower in the brain than in the cord. It is worthy of note as bearing on this last point that according to Watson ('03, p. 91 and 105) medullated fibers in the spinal cord of the rat are first found on the second day after birth, while in the cerebrum, they are not found until the eleventh day. At that age—eleven days—the percentage of water in the brain has fallen to that of the cord at the second day, and it thus appears that the medullation of axones begins in both divisions of the central nervous system when these have acquired the same percentage of water.

This suggestion, that the onset of medullation is closely related to the percentage of water in the axones, fits with the common observation that the fibers first medullated in any locality become the largest (because they have the longest time to grow after reaching the condition in which they can become medullated) and that in any nerve containing medullated and non-medullated fibers, it is the smaller (or younger) fibers which lack the sheath (Boughton '06). Also, as the portion of the axone nearest the cell body is the older, and hence would have the lower percentage of water, this should be the portion first medullated; a conclusion which fits with the observations.

It is hardly necessary to remark that these last two facts when

interpreted in this way, constitute indirect evidence for the view that the axone is an outgrowth from the cell body.

The medullation process as such does not reduce the percentage of water. This statement, already made in the "fourth" conclusion on p. 19 is here repeated because there is a more or less widely diffused opinion that the medullary sheath is a structure containing less water than the axones, and that it is the addition of the myeline, as it appears in the medullary sheaths, which largely serves to reduce the percentage of water in the white substance, and thus in the entire mass of the central system. For this there is no evidence. Charts 4 and 5, exhibiting the increase in the weight of water with increasing brain weight and cord weight, show no changes in the increment of water which would warrant such an explanation. It appears most probable therefore that the medullary sheaths when first formed have approximately the percentage of water characteristic for the axones at the time of their myelination, and after that, in company with the axones, they undergo a slow but steady diminution in water content.

A few separate determinations of the percentage of water have been made by various investigators on the white and gray substances of man and other larger mammals. These show without exception that between birth and maturity there is a greater loss of water in the white substance than in the gray. In these cases of course the white substance at maturity is always medullated, and thus the results do not answer the question whether the formation of the medullary sheaths has contributed to the diminution in the percentage of water. That the axones previous to medullation do show a reduction in the percentage of water with advancing age, is indirectly indicated by my own observations in the following way:

At birth (i. e., 5 grams body weight) the average percentage of water in the rats' brains of both sexes is 87.8 per cent (table 1). At eleven days of age, as shown in chart 2, it is about 84.8 per cent, a loss of three units, yet it is not until after the eleventh day that medullation in the brain begins. The percentage of water in the brain therefore falls off before medullation begins, and the nerve substance, cell bodies and axones, are the portions in which this diminution chiefly occurs.

As there is every reason to think from what we know concerning the relatively small reduction in the gray substance that the percentage of water in the cell bodies in this case is not progressing more rapidly than it does in the entire brain, it follows that in the remaining nerve structure—the axones—the percentage of water is reduced at least an equal amount. Since however the diminution in the percentage of water is found to be much greater in the mature white substance than in the mature gray, it seems probable that the axones are subject to a more extensive reduction in the percentage of water than are the cell bodies.[3]

From this it follows that the greater proportion of gray substance in the brain would tend to maintain in that organ a higher percentage of water at maturity, and the lesser proportion of gray in the cord, a lower percentage (see the measurements on the areas of the gray and white matter in the spinal cord as given by Watson, '03, p. 101).

But there is still one more peculiarity of the spinal cord which is important in this connection. This I have called (Donaldson '09, pp. 166–167) *passive lengthening*. The segments of the cord, especially in the thoracic region, undergo during growth a lengthening which is largely passive, and which does not imply any marked increase in the structural complexity of the cord, but serves mainly to keep the spinal nerves nearly opposite to their intervertebral foramina.

In the course of this lengthening, we have evidence that the volume of the gray substance is but slightly increased, while the proportions of the gray column are much modified in the sense that the diameter alters but slightly (it may even diminish) while the length is correspondingly increased (see the measurements on the areas of the gray and white matter in the spinal cord of the rat, Watson '03, p. 101, and Donaldson and Davis '03).

At the same time that this is occurring in the gray columns, the white tracts not only lengthen (passively) but also increase in the area of their cross sections, and thus at the end of any step

[3] The question whether a growing fiber at any age of the animal becomes medullated as soon as its percentage of water falls below the value at which medullation first begins after birth, cannot at the moment be answered. It is conceivable however that with advancing age this critical point for medullation is lowered.

in this transformation, we find a larger proportion of white substance than at the beginning. The white substance having a lower percentage of water than the gray, tends of course to bring down the general average. We know from previous studies that in the albino rat the weight (and length) of the spinal cord increases so long as the animal grows (Donaldson '09).

It is therefore the relative increase in the white substance due to this continuous passive lengthening—which is so marked in the cord—that can justifiably be held responsible for the more rapid decrease in the percentage of water in the cord after maturity.

In brief then, the more rapid diminution in the percentage of water in the cord up to maturity, and the greater rate of diminution after maturity, are due, aside from the excess of supporting tissues and vessels, to the greater amount of axone substance in the cord and the peculiar form of growth designated as passive lengthening.

General significance of this change. If we are correct in concluding that in the percentage of water we have a character correlated very closely with the age of the animal, and but slightly influenced by the conditions which modify general growth, it follows that this change must depend on processes intimately associated with the span of life or longevity of the animal concerned.

Broadly speaking, the changes in the percentage of water indicate progressive chemical modifications which take place in those constitutents of the cell that are most stable.

A comparison of the albino rat with man in respect to the percentage of water in the brain. In connection with such a comparison, I have examined the entire literature on the percentage of water in the nervous system. This literature needs to be summarized, but for such a summary, this is not the occasion. Out of the data available, I have selected however the findings of Weisbach ('68) and of Koch ('09) to be used in the present instance, as from these we get the best series of determinations of the water in the human brain at different ages. The data from Weisbach are as follows:

He determined the percentage of water in each case for six different localities in the encephalon: (1) white substance (callosum); (2) gray substance (corpus striatum); (3) gyrus (white

and gray mixed); (4) cerebellum; (5) pons; (6) medulla oblongata.

For the percentage of water in the human encephalon at birth, the determinations for these several localities are averages from three male and five female newborn infants.

A series of tests applied by me to Weisbach's data for mature brains have shown that the percentage of water in the entire encephalon is approximately equal to the sum of four times that in the white substance (1); five times that in the gray substance (striatum) (2); and once that in the cerebellum (3) divided by 10.

This procedure gives for the percentage of water in the human brain at birth 88.34 per cent. The value thus obtained is probably nearly correct.

By the same procedure I obtained from Weisbach's data for children between three and fourteen years of age (2 males: 3 years and 8 years; 2 females: 4 years and 14 years) a mean value for the entire encephalon of 79.2 per cent at 9.5 years. Finally, Weisbach's records for 64 males and 17 females, 20–30 years of age, give a mean value of 77.0 per cent.

Turning now to the determinations of Koch ('09) we find his average determination of the percentage of water in five human encephala at maturity to be 77.8 per cent. In a female brain of two years, he gives the percentage of water in the cortex of hemispheres as 84.49 per cent and in the callosum as 76.45 per cent.

In a male of 19 years, the cortex was found to have 83.17 per cent and the callosum 69.67 per cent. This last case may be taken to represent the conditions at maturity. This being assumed, it was found that combining the determination for the cortex in the proportion of 603 times to 397 times of that for the white substance gave a mean percentage of 77.8, which is Koch's determination for the water in the entire encephalon at maturity.[4]

Using the same proportions as those just given for the gray and white substances at maturity and applying them to the data for the brain at two years, mentioned above, we obtain as a mean

[4] The proportional abundance of the gray and white substance in the encephalon is not to be inferred from the numbers given above. Each investigator has used more or less arbitrary criteria for the gray substance, and a treatment of the results of an author in the manner here followed, has a value for the determinations by that author alone.

value for the percentage of water in the encephalon at two years 81.1 per cent. Thus we are able to obtain approximate values for the percentage of water in the human encephalon at birth, two years, nine and one-half years and at maturity, twenty-five years.

TABLE 6.

Comparison of the percentage of water in the encephalon of man and the albino rat at corresponding ages

MAN		RAT	
Age Years	Percentage of Water	Percentage of Water	Age Days
Birth	88.3	87.7	Birth
2 years	81.1	81.3	26 days
9.5 years	79.2	78.6	115 days
25 years maturity	77.0	78.0	290 days

In order to compare these determinations, it is necessary to recall that the span of life in man is about thirty times as long as that in the rat, and if this relation holds throughout the life cycle, it follows that each determination for man is to be compared with that for the rat having one thirtieth the human age taken.

The data for the rat are based on the entries in table 2 giving the percentage of water according to age.

This table shows that when we compare brains of corresponding ages, the diminution in the percentage of water in the two forms has similar limits, and would be expressed by a curve of like form in both instances.

When we examine the records for other mammals, we find almost no determinations for the water in the encephalon at birth, but we do find determinations for this character at maturity, and the values are very similar to those for man and the rat. Remembering that the relative amount of white matter in the encephalon varies somewhat in different species, and must therefore modify this result, we reach the interesting conclusion that probably in all mammals we shall find approximately the same range in the percentage of water between birth and maturity, and that the loss of water in them occurs in the same manner but that the

time required for the successive steps is determined by the intensity of the growth process characteristic for each species. (Rubner '08 and '08a).

CONCLUSIONS

1. In the albino rat between birth and maturity, the percentage of water in the brain diminishes from 87.8 to 77.5 and in the spinal cord from 85.6 to 68.0. Table 1.

2. The progressive diminution of the percentage of water is a function of age and is not significantly modified by any conditions to which the animals have been thus far experimentally subjected.

3. The diminution in the percentage of water is most rapid during the first twenty-five days of life; the period at which the central nervous system is growing most actively.

4. The maturing of the axone substance is characterized by a greater diminution in the percentage of water than is the maturing of the gray substance.

5. Medullation begins when the percentage of water in the brain and cord has diminished to about 85.3 per cent (second day in the spinal cord; eleventh day, in the brain).

6. The process of medullation itself as indicated by the formation of the medullary sheaths, is not a controlling factor in reducing the percentage of water in the central nervous system.

7. The range and course of the diminution of the percentage of water in the brain are similar in man and in the albino rat. The rapidity of change agrees with the intensity of the growth processes in each of the two species, and is therefore about thirty times more rapid in the rat than in man. This point has not been tested for the spinal cord.

8. It is probable that the same limits in the percentage of water and the same course of diminution will be found to occur in other mammals.

9. The progressive diminution of the percentage of water in the central nervous system with advancing age, is to be regarded as an index of fundamental chemical processes, which take place in the more stable constituents the nerve cells.

These processes are but little modified by changes in the environ-

ment and taken all together constitute a series of reactions which express not only the intensity of the growth process in the nervous system, but also the span of life characteristic for any given species.

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PLEASURE, PAIN AND THE BEGINNINGS OF INTELLIGENCE

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The tendency of animals to repeat acts which result in pleasure and to discontinue or inhibit acts which bring them pain is a fundamental feature of behavior on the utility of which it would be superfluous to comment. But why do animals behave in this fortunate manner, and how did they come to acquire the faculty of so behaving? To our ordinary plain way of thinking it appears sufficient to say that a dog eats meat because he likes it, and that he runs away from the whip to avoid its painful incidence upon his integument. These acts are such natural and obvious things to do under the circumstances that to inquire why the animal does what it likes and avoids what is disagreeable may seem a sort of philosophic quibble which only a mind "debauched by learning" would think of indulging in. But a little consideration will show that we have here a real and very knotty problem, or rather set of problems, of the greatest importance to the student of genetic psychology.

There are few better illustrations of the modification of behavior through experiences of pleasure and pain than that afforded by the behavior of young chicks, which has been so well studied by Lloyd Morgan. A young chick when first hatched has the instinct to peck at all sorts of objects of about a certain size. If an object is a little too large the chick may hesitate. Should it venture to peck at the object and derive a pleasant taste from it the hesitation in the presence of similar objects becomes reduced and will finally disappear. If the chick in the course of its pecking seizes a caterpillar having a nauseous taste it is much less apt to seize a similar caterpillar a second time. The painful or un-

pleasant experience it derives in some way inhibits further action towards that class of objects.

We have in this modification of instincts through the pleasurable or painful effects they produce the beginning of intelligence. The pecking, swallowing, and avoidance of certain objects are purely instinctive acts based on the chick's inherited organization. After its first experiences with pleasant or nasty caterpillars the chick is a different creature; it has learned by experience; and henceforth its acts, which at first were in a general way adaptive, become more perfectly adapted to its needs as the result of its learning. Instinct supplied the impetus to action and in a measure determined the direction of action, but intelligence refines upon the instinctive behavior and effects a closer adjustment to the environment.

In lower forms associations are formed as a rule with great slowness. Behavior is almost entirely instinctive, and the organism can be made to deviate from its stereotyped methods of action only with difficulty. It is probable that in low forms where associations of only the simplest kind can be established there is no association of ideas involved; and in fact there is no conclusive evidence of the existence of ideas even in animals quite high in the scale. Most animal learning consists in forming associations between certain sense experiences and certain actions which bring pleasure or pain. A common way of teaching an animal a trick is to try in various ways to induce it to perform the desired action and then to reward it by food or some other means of giving it pleasure. In this way the connection between the situation and the act is reinforced and the act follows more readily when the animal is placed a second time under the same conditions.

Consider the case of a cat placed in a box which can be opened by pressing down a lever or pulling a string, as in the experiments of Thorndike. If the cat is hungry and food is placed outside, the animal will probably make a vigorous effort to escape by clawing and biting in various parts of the enclosure, which are the usual instinctive methods employed in similar situations. If the right movement is hit upon and the cat gets out and secures food, it will probably make its escape more readily than before

when placed in the box a second time. After a number of trials the cat will come to make the right movements for escaping very soon after being placed in the box and its various useless random movements will be discontinued. The connection between the perception of the mechanism of escape in the box and the act necessary to gain its liberty comes to be more and more firmly established in the cat's brain with repeated experiences. The cat perceives a number of things in the box and performs a number of different acts but out of all these, associations are formed only between certain stimuli and those responses to them which bring pleasure to the animal.

Pleasure and pain therefore have apparently a fundamental connection with the development of intelligent responses out of instinctive activity. Were there not something to clinch or strengthen the connection between certain stimuli and the appropriate responses to them the organism might perform random movements till doomsday without being a whit better off. It is a problem therefore of fundamental importance to ascertain in what the mechanism of this ability to profit by experience essentially consists. It is not mere habit, not the mere making more permeable certain preformed connections in the brain. One act would then be just as apt to be followed up as another. Whether an act tends to be followed or not depends on what it brings to the organism. Apparently we have to do with a selective agency which preserves or repeats certain activities and rejects others on the basis of their results.

The importance of random movements lies in the fact that they offer opportunities for making favorable adjustments. For the development of intelligence they play a similar rôle to that of variations in the process of evolution. The animal that does the most exploration is the one most likely to hit upon new advantageous adjustments. In the same way intelligent adjustments as James has contended are favored by a multiplicity of instincts, especially if these instincts are of a contrary or conflicting nature, for now one and now another instinctive tendency may be reinforced in different conditions to which each may be adapted. Instinctive fear may be modified through experience so that it is

no longer attached to objects that are found to be harmless, while it may be intensified in relation to other objects that are found to be sources of injury. Where there is hesitation between the exercise of two instincts such as the tendency to pursue an animal as prey, and the instinctive fear which that animal may awaken, experience may quickly point out which proclivity is the more advantageous to follow. The pleasure-pain reaction enables an animal to select, so to speak, out of its stock of instinctive endowments those responses which are best adapted to the particular situations that confront it. It is a means of adapting instincts to new or inconstant conditions and thus of effecting a closer adaptation to the environment than that which would be possible by following purely congenital modes of response. The development of the pleasure-pain reaction marks one of the most important steps in the evolution of behavior, for the entire superstructure of intelligence in all its stages is based upon it, and it is not surprising that many writers regard it as an index of the beginning of consciousness, a point where a new entity is somehow mysteriously injected into the universe.

It is a general rule that what is pleasant is beneficial and what is painful is injurious; and, therefore, by following its desires and aversions an animal is guided in a tolerably safe course. Eating when hungry, drinking when thirsty, seeking warmth when cold, exercise when in a state of vigor, and rest when fatigued, all bring a state of satisfaction or pleasure. On the other hand, eating and drinking after a certain stage of repletion has been reached, or attaining too great a degree of warmth may be positively painful, the pain being correlated with carrying on these activities until they become injurious to the organism.

But it is well known that this correlation of the pleasant with the beneficial is not an absolute one. With complex creatures like ourselves with a multitude of different propensities and interests it is not infrequent that the pursuit of what is agreeable leads to all sorts of unfortunate consequences even of a purely physiological nature. In the lower animals where pleasure is a safer guide than among ourselves, what is pleasant is not always what is organically good. Poisonous articles may be eaten with appar-

ent relish and alcoholic liquors are readily imbibed even by such primitive creatures as bees and wasps upon their very first acquaintance with these intoxicants. But aside from exceptional cases pleasure in the animal world is a sufficiently good index of what is beneficial that under conditions which ordinarily present themselves it seldom leads to injurious courses of action.

The relation between the pleasant and the beneficial is, however, probably not a primary one, and it is not improbable that it represents a connection established by natural selection, as was first maintained by Herbert Spencer.

> If the states of consciousness which a creature endeavors to maintain are the correlatives of injurious actions, and if the states of consciousness which it endeavors to expel are the correlatives of beneficial actions, it must quickly disappear through persistence in the injurious and avoidance of the beneficial. In other words, those races of beings only can have survived in which, on the average, agreeable or desired feelings went along with activities conducive to the maintenance of life, while disagreeable and habitually-avoided feelings went along with activities directly or indirectly destructive of life; and there must ever have been, other things equal, the most useful and long-continued survivals among races in which these adjustments of feelings to actions were the best, tending ever to bring about perfect adjustment.

This explanation which has become widely accepted leaves a fundamental question unanswered. It does not explain why certain acts are stamped in and certain others stamped out. Of the mechanism of this process, which is the real problem involved in the pleasure-pain reaction, we are as ignorant as before. The explanation means that animals which took pleasure in following acts that brought them benefit were preserved and those that did not behave in this manner were eliminated. But why does an animal tend to repeat an act that brings it pleasure and avoid one that produces pain? It seems so natural for creatures to behave in this way that the existence of any problem here is usually unsuspected, but this is the problem that confronts us when we endeavor to obtain a clear understanding of the way in which intelligence develops out of instinct.

In the pleasure-pain response we have two problems of a quite

different nature: (1) the problem of how behavior is modified by its results, and (2) the problem of why pleasure is associated with certain physiological activities such as securing movements and pain with others such as avoiding movements. The latter problem is one whose solution appears holpeless. If we accept the doctrines of psycho-physical parallelism in any of its forms, we must deny that psychical states are, strictly speaking, the causes of physical changes. Why then should pleasure be connected with one kind of activity and pain with another? Why not just the reverse? This problem is, I believe, insoluble, because it is a question of the relation of the physical and the psychical; it is of essentially the same nature as the question why one kind of retinal stimulation produces a sensation of red and another a sensation of green. Physical and psychical states are correlated in particular ways; this we accept as a matter of observed connection. But why a certain kind of brain vibration is associated with a state of consciousness we call a sensation of red instead of some other state is a question upon which we may intend our minds indefinitely without the least profit. If we adopt any other theory of the relation of mind and body we are in no way better off. If we have to do with a preordained connection of pleasure with certain physiological activities and pain with certain others, this connection is no more intelligible if we admit the interaction of psychical and physical states than it is under the theory of parallelism. We can only say that such is the observed relation of the phenomena. It may be regarded therefore as a piece of good luck that we are constituted so as to pursue pleasure and avoid pain. We might have been endowed with a fatal tendency to do just the reverse. Pain instead of pleasure would then have been the correlate of physical well being; those forms in which the painful corresponded with the organically good would have been preserved, the others would have perished; and thus there would have been established a correlation between what is sought and what is conducive to organic welfare, as there is now, but of a quite different kind.

There is another way of looking at the problem which avoids the difficulty we have mentioned; and that is to suppose that pleasure

and pain are such only through their motor correlations. We may say, not that pain is mysteriously associated with withdrawing activities, but that pain is the psychic state which accompanies such activities—that it is those activities that constitute it pain. Pleasure would thus become the feeling which is reached out for; pleasure is pleasure just because it is sought. Or to state the matter somewhat paradoxically, if we were so constituted as to seek pain, the pain would not be pain but pleasure. It is indeed difficult to define the difference between pleasure and pain without appealing to the motor accompaniments of these feelings. The following words from Spencer are significant in this connection:

> If we substitute for the word Pleasure the equivalent phrase—a feeling which we seek to bring into consciousness and retain there, and if we substitute for the word Pain the equivalent phrase—a feeling which we seek to get out of consciousness and to keep out, etc.,

showing clearly that the qualities of these feelings are defined in terms of their motor accompaniments.

If we sought pain with quickened pulses and enthusiastic efforts, smiled when we obtained it, endeavored to keep it in consciousness as long as possible, and acted in all ways towards it as we now do towards pleasure, would it not then be pleasure? Such a conclusion, I must confess, jars against ones common sense habits of thought. Would it not seem absurd to say that red would be blue and blue red if the motor tendencies, whatever they may be, which these sensations awaken, were reversed? Would not the sensation of pain be the same if our nervous system were so constructed that excitations producing pain led to entirely different activities? How far the qualities of sensations are to be regarded as things standing as it were by themselves, just as atoms are supposed to possess certain qualities independently of their various combinations, may be open to question. It is a question for which fortunately our general discussion does not require an answer and we may leave it therefore to specialists in psychology and metaphysics. What we are concerned with at present is an explanation of the pleasure-pain process. If we

can explain this in physiological terms we can safely leave the preceding question to one side to be answered in whatever way it may.

Turning then to the problem of how behavior comes to be modified in adaptive ways by the pleasureable and painful experiences it brings to the animal, it is evident that it can be treated as a problem of physiology. We are dealing with a series of physiological reactions and how they come to be modified. We may assume that psychical states enter into the chain of causes and effects that make up an animal's behavior, but it is not clear that such an assumption throws the least light upon our problem, and it is open to serious objections on both scientific and metaphysical grounds. We shall therefore consider the question purely from the physiological standpoint. Viewed objectively we find that in an animal's behavior certain acts when once performed tend to be performed with greater readiness under similar conditions a second time, while other acts once performed tend under similar conditions to be inhibited. This problem of learning, Baldwin observes "is the most urgent, difficult and neglected question in the new genetic psychology." Spencer with his characteristic insight into fundamental problems has grappled with it and has attempted to give a physiological explanation. Pleasure, according to Spencer, is the concomitant of heightened nervous discharge; pain the concomitant of lessened discharge. In an animal with a diffuse discharge of its nervous energy resulting in random movements, some of these movements bring a heightened nervous discharge with its psychic accompaniment of pleasure. This tends to reinforce the movement that brought the increase of nervous energy and to cause it to be repeated. Responses resulting in pain tend on account of the diminution of nervous discharge that follows to be discontinued, and in this way the organism is kept repeating certain acts and avoiding others.

"Along with the concentrated discharge to particular muscles," says Spencer, "the ganglionic plexuses inevitably carry off a certain diffused discharge to the muscles at large, and this diffused discharge produces on them very variable results. Suppose, now, that in putting out its head to seize prey scarcely within reach, a creature has repeatedly failed. Sup-

pose that along with the group of motor actions approximately adapted to seize prey at this distance, the diffused discharge is, on some occasion, so distributed throughout the muscular system as to cause a slight forward movement of the body. Success will occur instead of failure; and after success will immediately come certain pleasurable sensations with an accompanying draught of nervous energy towards the organs employed in eating, etc. That is to say, the lines of nervous communication through which the diffused discharge happened in this case to pass, have opened a new way to certain wide channels of escape; and, consequently, they have suddenly become lines through which a large quantity of molecular motion is drawn, and lines which are so rendered more permeable than before. On recurrence of the circumstances, these muscular movements that were followed by success are likely to be repeated: what was at first an accidental combination of motions will now be a combination having considerable probability."

Bain's view of learning is much like that of Spencer.

We suppose movements spontaneously begun, and accidentally causing pleasure; we then assume that with the pleasure there will be an increase of vital energy, in which increase the fortunate movements will share, and thereby increase the pleasure. Or, on the other hand, we suppose the spontaneous movements to give pain, and assume that, with the pain, there will be a decrease of energy, extending to the movements that cause the evil, and thereby providing a remedy. A few repetitions of the fortuitous concurrence of pleasure and a certain movement, will lead to the forging of an acquired connection, under the law of Retentiveness or Contiguity, so that, at an after time, the pleasure or its idea shall evoke the proper movement at once.

The theories of Bain and Spencer are discussed in detail by Baldwin, who, while differing from these writers in certain points which need not here be dwelt upon, adopts essentially the same view as regards the mechanism of reinforcement and inhibition. With Bain and Spencer, Baldwin assumes that

the pleasure resulting from the first accidentally adaptive movement, issues in a heightened nervous discharge toward the organs which made the movement, a discharge which finds its way to the same channels as before, and so makes it likely that the same movement will be repeated, the external conditions remaining the same. . . . . Pleasure and

pain can be agents of accommodation and development only if the one pleasure, carry with it the phenomenon of "motor excess," and the other, pain, the reverse—probably some form of inhibition or of antagonistic contraction.

The theories of Spencer, Bain and Baldwin are physiological since they attempt to explain the modifications of behavior, not through the influence of certain states, but as the effect of the physiological conditions of which these states are the concomitants. The theories are all open to the objection that pleasure is by no means the constant concomitant of heightened nervous discharge. Laughing and crying are very similar in their physiological expression though they go along with very different psychic states. A child who burns his hands and writhes about in agony certainly manifests a heightened nervous discharge, but he shows no tendency to put his hands again into the fire. Another outreaching movement of the child brings his hands towards a pleasant degree of warmth. The movement tends to be repeated. The nervous discharge in the first case is much greater than in the second, but in both cases it goes to the arm, though along somewhat different nerves. It is obvious, I think, that we cannot account for the difference between the responses to pleasurable and painful stimuli on the basis of any quantitative difference in the discharges to the part affected. It is a matter of nervous connection rather than quantity of nervous energy.

Pain-giving stimuli, owing to the arrangement of an animal's reflex arcs, are generally followed by a withdrawing movement of the part stimulated, but that there is a tendency for the "increased energy of the pleasure process" to flow "into the channels of the movement associated with the pleasure" (that is, I take it, the movement which brings pleasure) is by no means evident. There is, I think, no primary tendency, as Spencer and Bain seem to think, for the nervous discharge to take the direction of the organ from which the pleasure is derived. Animals, it is true, move so as to bring an organ which is pleasantly stimulated again under the action of the stimulus, but this is often due to the discharge going mainly to a quite different part of the body, such as distant appendages, instead of the part directly affected.

The theory of heightened nervous discharge as expounded by Spencer, Bain and Baldwin, fails to give us, I think, the desired explanation of the acquirement of individual accommodations, and one naturally turns to other theories of the psycho-physiology of pleasure and pain for light. Here, however, we are led into a veritable quagmire of psychological speculation, for there are few fields in which there are so many and so fundamental differences of opinion among competent psychologists.

It is quite generally agreed that there are specific pain sensations aroused by the stimulation of special nerve endings. The existence of specific pleasure sensations is much less widely accepted. Granting the existence of pain sensations, the relation of these to other forms of pain is by no means clear. Stumpf has made an elaborate attempt to show that all pleasurable and painful feelings are really sensations, a conclusion which he shares with a number of other psychologists. Wundt, Külpe, Ebbinghaus, Titchener, and others, while admitting the existence of specific pain sensations contend that there is a class of feelings distinct from sensations, the affections. But among those who contend for affection as a distinct category of psychological phenomena, there is much lively controversy. Some contend, like Titchener, that "there are only two kinds or qualities of affection, pleasantness and unpleasantness." Wundt in his well known tridimensional theory of feeling, which has secured a small following, attempts to prove that feelings may differ in at least three pairs of contrasted attributes of which pleasantness and unpleasantness form one. Royce in his Psychology reduces these to two. The field of enquiry is one of peculiar difficulty and experiments in the hands of different investigators have yielded contradictory results. Brahn, for instance, finds pleasure and pain accompanied uniformly by certain variations of the pulse and reaches results confirmatory of Wundt's tridimensional theory. Titchener, Hayes and others, on the contrary, have reached results which they regard as clearly at variance with Wundt's doctrines.

There is no agreement among psychologists as regards the physiological expression of the pleasant and unpleasant. Féré, Leh-

mann, Mentz, Zoneff and Meumann find that pleasant and disagreeable states are quite uniformly accompanied by certain characteristic physiological processes. Among the accompaniments of pleasurable feeling we have increased amplitude of heart beat, a slowing of the pulse, dilation of peripheral blood vessels and an increase in the rate of breathing, accompanied by a decrease in its depth. Unpleasantness on the other hand is said to go along with quickening of the pulse, contraction of the blood vessels, and slower and deeper respiration. Other investigators, however, fail to obtain such uniform results. Kelchner finds that agreeable tastes have an opposite effect on the pulse from that produced by sounds and colors and that the respiratory changes corresponding to agreeable and disagreeable stimuli are far from constant. Shepard in studying the effect of stimuli upon the peripheral circulation finds that 19 agreeable stimuli gave a fall of volume distinctly, while 4 gave a possible rise; 15 disagreeable stimuli gave a distinct fall, and 2 a possible rise. Agreeable smells were found to deepen the respiration and disagreeable ones to have the opposite effect.

A disagreeably exciting sound or a noise tends to deepen breathing and often makes it irregular also. Agreeably exciting stimuli at least as often increase as decrease the depth.

The volume of the brain (studied upon a person who had lost a portion of the skull in an accident) showed no constant relation to agreeable or disagreeable stimuli, both producing in general an increased volume and increased cerebral pulse.

Shields has reached similar conclusions in studying the effect of odors upon the circulation. Heliotrope and wood violet were enjoyed by the subject experimented on, but the volume of the arm diminished quite as often as increased during their application. Indol and skatol are unpleasant odors, but the volume of the arm frequently increased during the first few seconds of their application and then decreased. The effects of odors were very different with different people, and with the same person at different times, and the author concludes that

the experiments give no support to the view that pleasant sensations are accompanied by a diminution of blood supply to the brain, and unpleasant sensations by the reverse effect.

Angell and Thompson express themselves as follows concerning the physiological concomitants of pleasant and unpleasant reactions:

> It is in the case of the emotions, where the agreeable and disagreeable experiences are most intense, that we should expect to find the most marked and constant correspondence of agreeable states with one set of physiological processes and of disagreeable states with an antithetical set, if any such relationship existed. But our curves show not the slightest evidence of such an interconnection. None of the various factors involved, vaso-motor level, rate and amplitude of the pulse curve, position and emphasis of the dicrotic notch, or rate and amplitude of the breathing, changes uniformly in one direction for agreeable experiences, and in opposite direction for disagreeable experiences. . . . . Almost all of our emotional experiences, whether agreeable or disagreeable, produced vaso-constrictions.

The results yielded by the study of affective states, by means of instruments for recording changes in circulation, pulse, and respiration and other physiological manifestations do not afford at present a very encouraging outlook for the solution of our problem, for in so far as pleasurable and painful experiences are not associated with uniform outward expressions it is difficult to obtain clear evidence of the accompanying internal physiological states. It is commonly assumed that there is something in pleasure and pain or their physiological correlates that reinforces or inhibits, as the case may be, the responses from which these states result. What this something is and how it produces its effects are problems for which a satisfactory solution has not been offered. There has been a bewildering variety of theories of the nervous correlates of pleasure and pain and of pleasantness and unpleasantness but there has been little attempt to apply these theories to explain the mechanism of profiting by experience, and it is difficult to see how most of these theories would help us in regard to this matter even were they established.

A new point of view in regard to our problem has been presented by Hobhouse in his Mind in Evolution. To illustrate this view let us recur to our chick. When a nasty caterpillar is seen for the first time the visual stimulus sets up a pecking reaction. This is followed by the stimulus of a bad taste which sets up various rejection movements, such as ejection of the food and wiping the bill. The order of events is

stimulus. . . . pecking. . . . bad taste. . . . rejection.

When the same kind of caterpillar is met with a second time the stimulus tends to elicit the rejection movements with which it has been associated instead of the movements of pecking. Is not the inhibition due to the fact that the stimulus has become associated with a response which is incongruous with the first? Movements of rejection and avoidance are incompatible with those of pecking and swallowing and it may therefore be unnecessary to look to any peculiarity of the physiological correlates of pain for an explanation of the inhibition of the original reaction. The stimulus becomes coupled with a new reflex arc; nervous energy is drained off in a new channel, and the future behavior becomes changed. If the taste is a very bad one, a great deal of energy is involved and the connection with the rejection response made very permeable and the rejection movement easily set up. If a person is confronted with a sight of some nauseating medicine he has recently taken, avoiding or rejection movements are set up, such as making a face or even retching movements of the stomach. Is it not these movements or attempts at movements that really inhibit the taking the medicine? This is evidenced by the chick described by Lloyd Morgan, which after an experience with a nasty caterpillar approached one a second time but stopped and wiped its bill and went away as if it actually repeated its first experience. Of course inhibition of the original response does not always involve contrary movements but there may be impulses to such movements which do not issue in action. The principal feature in the modification of action through painful experiences is the assimilation of impulses incongruous with the original one.

In the reinforcement or stamping in of a reaction to a particular stimulus that brings pleasure, it certainly seems as if pleasure or its physiological correlate in some way serves to cement more firmly the association between the stimulus and the response. Let us consider, however, the case in which the chick pecks at a caterpillar which has a good taste; the presence of the caterpillar in the mouth excites the swallowing reflexes; in the presence of a similar caterpillar the pecking response is made more readily than before and whatever hesitation there may have been at first disappears. Is not the difference from the pain response due to the fact that there is an organic incompatibility between the first and second responses in the pain response, while there is an organic congruity or mutual reinforcement of these responses in the other? Pecking and swallowing form the normal elements of a chain reflex; when one part of the system is excited it tends to excite the rest, to increase the general tonus of all parts concerned in the reaction. Many reflexes instead of being mutually inhibitory, tend to reinforce one another's action. According to Sherrington,

> When in the spinal animal the one fore foot is stimulated, flexion of the hind leg of the crossed side is often obtained. Stimulation of that hind foot itself also causes a like reflex of that limb. When these two are concurrently stimulated, the flexion movement is obtained more easily than from either singly. These widely separate reflex-arcs therefore reinforce one another in their action on the final common paths they possess in common. Similarly with certain reflex-arcs arising from the skin of the pinna of the crossed ear. In them excitation reinforces that of the just mentioned arcs from the fore foot and opposite hind foot.

The presence of savory food in a dog's mouth causes the secretion of saliva and the movements of chewing and swallowing, and the stomach at the same time may be stimulated to secrete gastric juice. These activities are organically associated and they are usually preceded by seizing acts of various kinds. A particular object, then, which evokes the seizing response and which is of a character to set up these other reactions becomes more readily responded to again. The seizing reaction becomes assimilated to the other reactions which dispose of the food.

Let us illustrate this view by the results of some experiments on the crayfish. If a piece of meat is placed a short distance in front of a crayfish, the first response is usually a slight twitching of the outer ramus of the antennules; this is followed by chewing movements of the mouth parts and restless movements of the legs; the small chelipeds are moved back and forth and grasping motions are made by the small pincers as if in the endeavor to find some object. These movements may be followed by walking, and exploring movements of the large chelipeds. The coördinated movements of the antennules, mouth parts and legs may be regarded as a complicated form of chain reflex. If now we apply a stimulus to any of the organs concerned, it tends to set up the reflexes in the rest. If a drop of meat juice be applied by means of a capillary pipette to the tip of a small cheliped the first response is usually a twitching movement in the chela followed by an exploring movement of the limb. This is followed by similar movements of the other chelipeds and chewing movements of the mouth parts, and these by the twitching of the outer ramus of the antennules. If the stimulus be applied to the maxillipeds the chewing movements of the mouth parts are followed by the movements of the antennules and the legs. Any reflex element in this chain of reflexes involved in food taking tends to set off all the others.

I have trained crayfish, by feeding them by hand, to come toward me to get meat. At first I would very slowly bring a piece of meat held in a fine forceps near the antennules. After the movements of the antennules and mouth parts the grasping movements of the chelipeds would result in securing the meat. After some trials I would not allow the meat to be pulled away from the forceps until the crayfish struggled awhile to secure it; at the same time I moved my hand about so as to accustom the animal to my movements. There is a struggle between the instinct to flee from a large moving object and the instinct to secure a savory morsel which has been seized. With careful management the latter instinct may be made to predominate over the former and gradually the fear of one's movements becomes much reduced. The crayfish finally came to associate the approach of my hand with being fed and would rear up and hold out its large chelae,

much as in the ordinary posture for defense. Crayfish and crabs often assume this attitude while alarmed and retreating from danger, but the crayfish would come toward my hand and moreover would react in this way only when hungry, so that the response was not a fear reaction but one showing rather the absence of fear. One individual would greet me as I entered my room in the morning by raising up its chelipeds and coming toward me, and it would follow me about as I went from one side of its inclosure to the other. When fed, however, it would manifest no further interest in my movements. I had come to mean food and was responded to, I fancy, much as the crayfish would respond to a small object of prey which it could approach without fear. A part of the crayfish's congenital endowments is the instinct to approach small moving objects, as it is to flee from large ones. This instinctive reaction to small objects normally precedes the movements of seizing and devouring, just as the latter are preceded, though more uniformly, by twitchings of the antennules; the two sets of processes are parts of a normally associated chain of events.

The appearance of a large moving object has been associated with a mode of response (food taking) which is incongruous with the fear response; the latter is therefore inhibited. The reaction proper to the small object normally pursued has become joined to the food taking activities and the large object is followed. The large object may mean food, but there must be supplied an impulse to go toward the object. This probably comes, not from a fiat of the creature's will, but from an already existing instinctive proclivity to pursue objects of possible interest or food.

According to the view here presented, whether a particular response to a stimulus tends to be repeated more readily or discontinued, depends not upon the peculiar physiological state which may be produced in the brain, but upon the kind of responses which the stimuli brought by the act call forth. If an outreaching reaction becomes coupled with a withdrawing response the result is inhibition. If the reaction, on the other hand, brings stimuli which produce congruent reactions the association formed with these latter reinforces the first reaction. The pleasure-pain

response then resolves itself into the formation of associations. Withdrawing and defensive responses are usually initiated by pain giving stimuli and the instinctive or random movement which brings a painful stimulus is inhibited under similar conditions in the future, not because of the pain of its physiological correlate but because it comes to be associated with a withdrawing or defensive and hence an incongruous or inhibitory reaction. Pleasure and pain thus interpreted have no mysterious power of stamping in or stamping out certain associations. Whether the result is reinforcement or inhibition depends on the way in which a reaction and the secondary responses resulting from the situation in which the organism is thereby brought, happen to harmonize.

The step from instinct to intelligence viewed as a physiological process involves, therefore, no essentially new element beyond the well known physiological properties of the nervous system, and we are not committed to any particular hypothesis as to the physiological accompaniments of pleasure and pain, or pleasantness and unpleasantness, in order to understand how behavior may become adaptively modified. How far the interpretation given will enable us to explain the development of intelligence I do not pretend to say. It may break down in attempts to apply it to higher forms of learning, but it affords a useful working hypothesis and takes us a way, I think, towards the solution of our problem.

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THE FORMATION OF HABITS AT HIGH SPEED[1]

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From the Zoölogical Laboratory of the University of Michigan

WITH TWO FIGURES

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INTRODUCTION

The method by which habits are ordinarily educed consists essentially in presenting a problem whose solution depends on the slow, and often painful suppression of irrelevant actions, and the survival of only those that count. The results so achieved are invaluable, but from the nature of the case difficult to verify. So much time is required that students of all classes are apt to be told in words, rather than by actual experiments, what has been accomplished in this interesting field. Habits formed slowly and gradually are not only incapable of quick demonstration, but the "slow method" leaves altogether untouched, a wide range of behavior. Animals do not always act slowly; they do not always overcome, with deliberation and care, the difficulties that block

[1] Directly, as well as indirectly, I am indebted to Miss Frances J. Dunbar, and to Miss Nina Gage, for many of the results on which the present communication is based.

the road to food and comfort; indeed, in nature there are many problems whose solution must be accomplished at once with the utmost rapidity, and to the swift alone is the race.

METHODS

The animals used were white rats of different ages, and the general method, a modification of the time-honored labyrinth. Instead of the usual form, however, I constructed a zinc tank, 2 feet, 1 inch square, and 6 inches deep, and covered it with coarse-mesh zinc netting, in the exact center of which a circular opening, fitted with a cylindrical shoot, serves as an entrance for the animals. At each of the four corners the cover, which is firmly clamped to the sides of the tank, has a small hinged door that can be opened at will, or made fast, likewise by means of clamps.

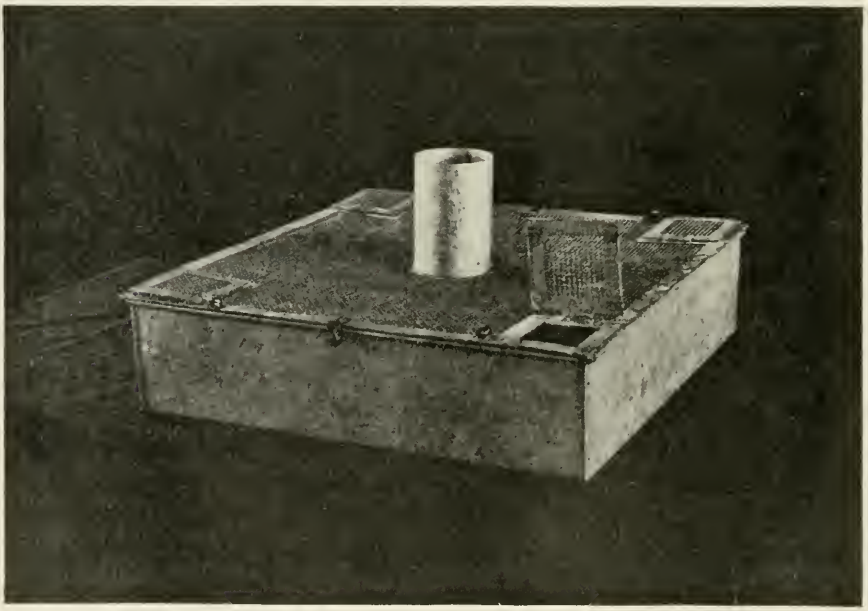

FIG. 1. Tank. Photograph by Miss Frances Dunbar. Dimensions 2 feet 1 inch square; depth, 6 inches; diameter of funnel, 4 inches; height of funnel, 6 inches; exits, 3 inches square.

During the experiments, the tank was filled, sometimes with warm, sometimes cold water; salt was added on some occasions, whereas on others additional stimuli were administered while the animals were falling through the shoot or after they had emerged through the open door. As there are four openings, any one of which may be made the correct one, and as the contents of the tank may be varied in many ways, and the experiences on entering and emerging complicated as much as one desires, the tank is in a real sense, labyrinthine, although simple in construction.

GENERAL RESULTS

It is needless to say that an inexperienced animal suddenly thrust into a tank of water makes strenuous efforts to escape. Under the conditions of the experiments it is not surprising that a high degree of variability should attach to the several attempts of an animal forced to undergo the experience of the tank half a dozen times in succession. Nevertheless there is an underlying regularity, for the time taken to escape in 83 per cent of the cases is less at the last attempt than at the first, whereas in 16 per cent it is greater, and in only 1 per cent unchanged.

Time records, while the most convenient form of registration, are nevertheless not the only ways in which the formation of a habit manifests itself. Very much to the point in this connection are tracings of the actual pathway pursued in escaping. Six such graphic representations are given in fig. 2, and show conclusively that the first turn of the path that led to the first escape occurs, often much abbreviated, and regardless of advantage, in nearly all of the succeeding trials.

In experiments into which so many complicating factors enter a regular and machine-like progress toward perfection cannot be expected, and its failure to appear is clearly shown in series A and B. Records C and D, however, illustrate distinctly how the path followed was simplified until in the last trials it became to all intents and purposes a straight line. Hand in hand with this was a reduction in the amount of time taken to effect the escape.

Two facts of considerable interest are to be read in these tracings. In series B, C, D, and E, the pathways are without exception dextral; in series E, on the other hand, all the turns made are sinistral. A directive factor seems to be operative, but analysis shows that it may not be simple. A considerable number of tests was made with blindfolded animals, and with young whose eyes were not yet open. The results in general indicate that certain individuals have a natural disposition to turn to the right, rather than the left, in swimming, whereas in others the reverse tendency is equally marked. I have made no experiments to determine whether this tendency is due to differences in the semi-circular

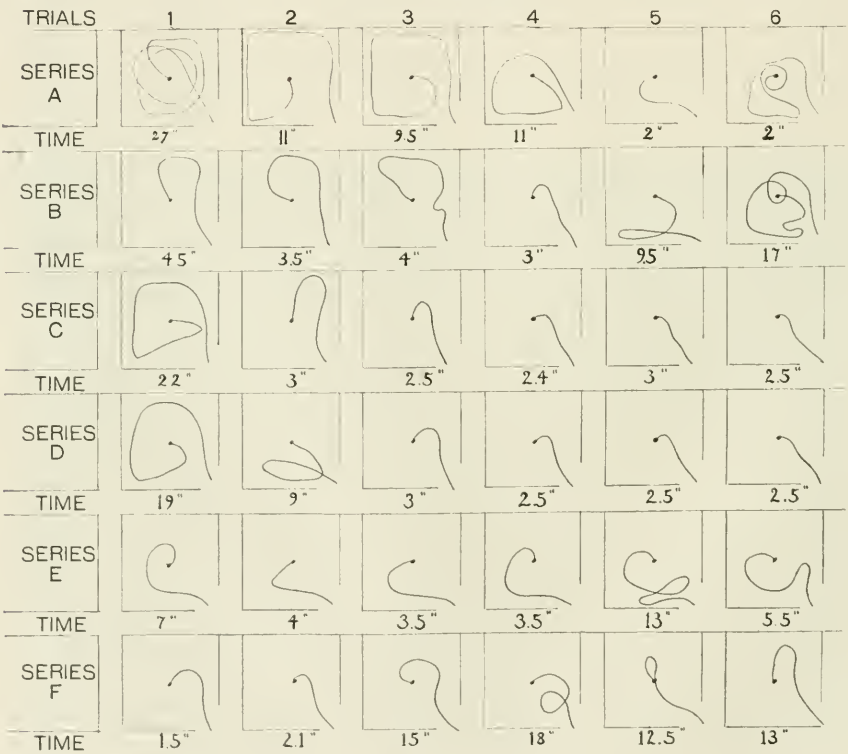

FIG. 2. Graphic representation of six sets of trials made by six individuals. The squares represent the tank, the incomplete corner, the open exit. Above the first series of records are figures indicating the number of each trial, whereas beneath the squares are the time records made in traversing the pathway indicated within each square. In every case the animal started from the central dot.

canals, or to inequalities in the right and left swimming musculature, or the neuro-muscular coördinations, or to a combination of these possible factors. Any one of them, or any combination, except of course a compensatory one, might be responsible for the fact that some animals naturally turn to the right, others to the left.

The determination of the true basis of this behavior would be very difficult because the repetition of the tests that bring it forth leads to the formation of a habit. That such becomes established, is, I think, sufficiently clear from the diagrams, as well as from the time records beneath them. Very interesting are series of which A is typical. In these, an animal, either because of its structure or its habit, turns consistently in one direction, in the present example, to the right. For some reason, exactly the reverse course was taken, with great success, at the fifth trial, and the sixth shows distinctly how this new departure complicated the course.

Series A illustrates a second fact, namely, that in many cases the pathway in succeeding trials becomes more complex, and the time consumed in escaping may increase. Nothing would be further from the truth than the conclusion that increase in either the complexity of the path or in the length of time, indicates progressive stupidity. The conditions of the experiments are such that a property analogous to cool-headedness in man, is at a premium. In many instances the animals became increasingly nervous, and lost their heads.

In problems whose solution can be effected, provided the animal has enough time and makes sufficient movements, this complication does not enter, but more than mere activity is necessary in a crisis. Animals which by luck or otherwise succeed early, behave much as they would in the face of "slow" problems; but animals that fail, or succeed only with difficulty, quickly become so handicapped by fear, and the useless activity characteristic of that state, that they fail more completely or succeed with much greater difficulty in later trials than they would in corresponding trials under more favorable conditions. While the experience of the tank brings out forcibly innate differences in the capacity to do the right thing quickly under stress, it is

hardly adequate to measure real differences in the ability to learn. The individuals which are thrown out of commission, by the very nature of the problem itself, are simply out of the running; they are unable to compete, and their failure is no more to be ascribed to inferior intelligence than the failure of a blacksmith to win prizes at a swimming meet is to be ascribed to the superior strength of the other contestants.

Objectively, of course, the facts are that some animals fail, whereas others succeed. Success may come with progressive reduction in the complexity of the path, and in the time taken to traverse it, or with either of these elements separately; failure may result from the corresponding opposites. We can say with justice that the capacity to learn also expresses itself in one or more of these ways, but the obvious inference would not be the correct one. Should the chain of activities that we are interested in be inaugurated, our objective measurements would give us the information we want, but when some other chain is set up, the measurements show simply the extent to which the second disturbs the first chain. In other words, if the problems convert the animal into a non-learning mechanism, the movements which the individual performs throw no light on its learning capacity. What they do show is, that under the given circumstances, some animals improve, others do not, but the capacity of those that fail remains unknown. Even those that succeed do not divulge all. Strictly speaking, their capacity is shown to be not less than the records indicate, but actually it may be considerably more.

The animals that improve emphasize a practical question of some importance. There are plainly two ways of solving the tank problem; by increasing speed, and by decreasing the length of the pathway. Cases in which these two go hand in hand, or in which constancy of speed is offset by an abbreviation of the pathway, offer no difficulty; but what shall we say when increased complexity of the pathway is compensated for by heightened speed? If we limit ourselves entirely to the objective time measurements, such an individual may seem to improve or to hold its own; if, on the other hand, we study the pathway alone, the animal is clearly loosing in fitness. The sixth trial in series A is a concrete illustration. How shall such an individual be rated?

As the objective records are at variance, it seems at first sight arbitrary to fix on either one or the other, and to say this is a measure of the truth.

Series C and D are from animals naturally well fitted to cope with the problem presented; natural fitness, however, varies, and some individuals inaugurate an overpowering set of altogether irrelevant, interfering movements. Such animals, practically, are not competing. Between these extremes most of the other individuals take their places, for their irrelevancies are not sufficient to destroy all chances of success, though marked enough to affect the general averages. The problem is to get out of the tank as quickly as possible, and is solved both by the animal that reduces the complexity of its path, either with or without an increase of speed, and by the animal that compensates inferiority in one direction by superiority in another. The latter might even win, but if we were offering prizes, justice would demand one for improvement in speed, the other for improvement in form. As form in the end makes for speed, not in individual cases, but on the average, the time records may be adopted officially as a practical, though not necessarily complete, measure of fitness.

SPECIFIC RESULTS

ADULT RATS

The first experiments were made with a tank differing slightly in size from the one described, and the animals, instead of being dropped into the water through a shoot that landed them in the exact center, were thrown in from one side, with a slight whirling movement. There was but one corner opening through which escape might be effected. The problem was thus essentially the same as the one described, and the differences in the records are mainly due to differences in the sizes of the two tanks, and to the way in which the animals were introduced. The whirling movement was adopted in order to insure that the effort to escape might not be begun in any except a chance direction. The results are tabulated below in self-explanatory form.

ANIMAL I.			ANIMALS II.			ANIMAL III.		
Trials	Time	Intervals	Trials	Time	Intervals	Trials	Time	Intervals
1	19.0″	0	1	14.0″	0	1	6.0″	1′30″
2	3.5″	0	2	11.0″	0	2	9.0″	1′10″
3	2.0″	1′	3	9.0″	1′	3	8.4″	1′30″
4	5.0″	15′	4	5.0″	0	4	26.0″	2′03″
5	3.5″	0	5	2.0″	15′	5	1.4″	2′00″
6	2.5″		6	2.9″	0	6	1.0″	Hours
			7	17.0″		7	1.4″	1′32″
						8	1.2″	1′20″
						9	1.6″	2′40″
						10	2.4″	0′40″
						11	2.0″	0′45″
						12	1.0″	

Characterization, Animal I: At first many trial movements, then stopped investigating. In the end did not hurry out. Averages: first three trials, 8.16″; second three trials 3.66″. Improvement, 55.1 per cent.

Characterization, Animal II: Accidental delay at second trial. Numerous extra movements in fourth. In seventh gave up trying to get out, hence this record is omitted in the averages. Averages: first three trials, 11.33″; second three trials, 3.33″. Improvement, 70.8 per cent.

Characterization, Animal III: Slight delay in starting watch at eighth trial. Averages: first six trials, 4.73″; second six trials, 1.60″. Improvement, 66.3 per cent.

ANIMAL IV.			ANIMAL V.			ANIMAL VI.		
Trials	Time	Intervals	Trials	Time	Intervals	Trials	Time	Intervals
1	57.4″	30″	1	55.2″	30″	1	13.1″	30″
2	3.2″	30″	2	7.1″	30″	2	18.1″	30″
3	8.2″	30″	3	5.1″	30″	3	4.3″	30″
4	12.3″	30″	4	6.3″	30″	4	5.1″	30″
5	8.2″	30″	5	5.2″	30″	5	5.2″	30″
6	8.2″	20″	6	15.3″	20″	6	3.3″	20″
7	9.3″	30″	7	3.3″	30″	7	12.2″	30″
8	4.4″	30″	8	6.0″	30″	8	13.1″	30″
9	4.3″	30″	9	12.2″	30″	9	22.3″	30″
10	3.2″	30″	10	19.2″	30″	10	8.0″	30″
11	3.1″	30″	11	4.0″	30″	11	31.4″	30″
12	5.0″		12	4.0″		12	4.3″	

Characterization, Animal IV: Animal quiet. Averages: first six trials, 16.25″; second six trials, 4.88.″ Improvement, 70 per cent.

Characterization, Animal V: Animal quiet. Averages: first six trials, 15.7″; second six trials, 8.11.″ Improvement, 48.4 per cent.

Characterization, Animal VI: Animal quiet in the first six trials, and apparently improving at a good rate. In the second set, however, it suddenly became "cranky" and did everything except the expected; it swam around aimlessly, or clawed the covering screen. Averages: first six trials, 8.18″; second six trials, 15.20″. "Deterioration," 85.8 per cent. This record is of necessity omitted in the later calculations.

Naturally there is much unevenness in these records, and at times the irregularities seem to obscure the evidence that a habit was formed, or to show that the exact opposite was established. Nevertheless there is a fundamental harmony beneath the discrepancies, and this is not destroyed even when the record of animal VI is admitted. For obvious reasons, however, it would be unfair to allow this animal to figure in calculations that involve the group of animals as a whole. On similar grounds I have excluded the 7th trial of animal II, for on that particular occasion this individual also very clearly did not attempt to escape. If with these modifications the results be taken as they stand, we can construct the following table for comparisons:

	FIRST HALF RECORD Averages *Seconds*	SECOND HALF RECORD Averages *Seconds*
Animal I	8.16	3.66
Animal II	11.33	3.33
Animal III	4.73	1.60
Animal IV	16.25	4.88
Animal V	15.70	8.11
General average	11.23	4.32

A glance at this record shows that experience, even when limited to very brief periods of intense activity, has its effect, and in the adults composing this group brought about on the average an increase of 6.89 seconds in speed, or an improvement of 62.1 per

cent. Considering the unfavorable conditions for the formation of a habit which these experiments present, the result is very marked.

YOUNG RATS

That this conclusion is not a mistaken one, is indicated by a similar treatment of the records of very young animals that underwent the experience of the tank. The individuals whose performances are tabulated in detail below were all from the same litter, and were aged three and a half weeks.

ANIMAL A.			ANIMAL B.		
Trials	Time	Intervals	Trials	Time	Intervals
1	9.0″	1′25″	1	5.0″	15″
2	4.0″	44″	2	13″	37″
3	5.0″	19″	3	14″	27″
4	2.5″	43″	4	10″	25″
5	5.0″	28″	5	4.5″	30″
6	2.0″	45″	6	5.0″	31″
7	3.0″	23″	7	7.0″	1′2″
8	1.3″	45″	8	3.5″	1′9″
9	1.5″	1′10″	9	4.5″	1′8″
10	1.0″	1′15″	10	2.0″	58″
11	2.0″	56″	11	5.5″	32″
12	2.0″		12	6.0″	

Characterization, Animal A: Animal between three and four weeks old; quick in its movements and not easily confused. Took a new route at the fifth trial, but at the sixth headed at once in the proper direction. Averages: first six trials, 4.58″; second six trials, 1.8″. Improvement, 60.7 per cent.

Characterization, Animal B: Animal between three and four weeks old; quick but irregular and not to be counted on. In the twelfth trial ducked under just before emerging, and lost considerable time. Averages: first six trials, 8.56″; second six trials, 4.75″. Improvement, 44.6 per cent.

Taking the figures as they stand, and comparing the average of the first half-record of each animal with the average of the second half-record, we get the following:

	FIRST HALF RECORD Averages *Seconds*	SECOND HALF RECORD Averages *Seconds*
Animal A	4.58	1.80
Animal B	8.56	4.75
Animal C	13.75	4.80
Animal D	6.43	4.54
General average	8.33	3.94

Here, too, is evidence that the behavior was modified by the "tank experience," but much more interesting is the fact that the changes undergone seem to harmonize very closely with the results secured by Watson ('03) under much more favorable conditions. The increase in the speed of the young animals was

ANIMAL C.			ANIMAL D.		
Trials	Time	Intervals	Trials	Time	Intervals
1	8.5″	42″	1	10.5″	24″
2	33.0″	22″	2	8.5″	35″
3	19.0″	20″	3	5.8″	1′10″
4	9.0″	34″	4	5.8″	40″
5	9.0″	36″	5	6.0″	47″
6	4.0″	53″	6	2.0″	56″
7	3.0″	1′	7	7.0″	40″
8	3.0″	1′4″	8	3.0″	53″
9	3.0″	43″	9	3.3″	1′10″
10	2.8″	1′7″	10	6.0″	58″
11	4.0″	1′28″	11	4.3″	42″
12	13.0″		12	3.5″	

Characterization, Animal C: Animal between three and four weeks old; very quick and nervous. In first trial explored all corners, became panic-stricken in the second, better in the third, but during the fourth lost much time clawing the wire screen. In the last half of the series the animal seemed to have found itself, and exhibited no semblance of fear. The length of the twelfth trial is due to having overshot the opening. Averages: first six trials, 13.75″; second six trials, 4.8″. Improvement, 64.4 per cent.

Characterization, Animal D: Animal between three and four weeks old. Relatively slow and indifferent. Averages: first six trials, 6.43″; second six trials, 4.57″. Improvement, 29.9 per cent.

on the average 4.39 seconds, for the adults 6.89 seconds; the former represents an increase of 53 per cent in efficiency, whereas the adults improved 62.1 per cent.

DURATION OF THE HABIT

That a habit formed under stress during brief periods of intense activity, may endure from fifteen minutes to several hours, the records already given show plainly. Special tests were made, however, to determine, if possible, whether any traces of the heightened efficiency might be found after several days. For this purpose, animals A, B, and C, of the preceding group of young were used, and adults A, B, C, D, E, and F, upon whose records fig. 2 is based. In the present connection I shall distinguish the three young animals by small letters, the six adults by means of capitals.

ANIMAL	DATE	TIME	TRIALS	AVERAGES
				Seconds
a	4/26	2.15 p.m.	6	4.58
a	4/26	3.28 p.m.	6	1.34
a	4/27	2.15 p.m.	6	4.69
b	4/26	2.30 p.m.	6	5.15
b	4/26	3.35 p.m.	6	4.75
b	4/27	2.30 p.m.	6	3.30
b	5/9	10.20 a.m.	6	5.65
b	5/9	10.45 a.m.	6	2.10
c	4/26	2.50 p.m.	6	13.40
c	4/26	3.43 p.m.	6	4.79
c	4/27	2.50 p.m.	6	7.00

The adults A, B, C, D, E, and F, whose records on January 15th, 20th, 22d, and 28th follow, were all used prior to December 11th of the year before, but unfortunately the earlier experiments were not performed under exactly the same conditions as those made in January, and a comparison of records separated in time by more than a month cannot be made. The results of the series separated in time by five days, by two, and again by six, can, however, be safely compared.

ANIMAL	DATE	DATE	DATE	DATE
	Jan. 15	Jan. 20	Jan. 22	Jan. 28
	Seconds	*Seconds*	*Seconds*	*Seconds*
A	10.40	1.66	1.91	2.08
B	6.90	10.25	5.83	6.00
C	5.90	9.08	13.75	10.50
D	6.41	5.66	6.08	6.08
E	6.08	32.82	11.41	9.75
F	15.75	19.00	16.00	10.16
General average	8.58	13.08	9.16	7.33

The results from the young animals are not especially conclusive. Taking the records as they stand, however, it may be said that two out of the three seemed to show the effects of their experiences twenty-four hours afterward, and one of the animals, after twelve days, very quickly recovered and in the end actually bettered its previous best record. It is to be expected, of course, that individuals vary greatly in the length of time which their habits, whether formed slowly or rapidly, endure; and furthermore, there is no way in which one can be reasonably certain, except by the method of multiple instances, whether, in the long run, animals without previous experience might not, on the whole, make as good records as those made by experienced individuals. As far as the group as a whole is concerned, it does not seem to bear markedly one way or the other on this particular question, although it does show that the final records of animals b and c were better than their initial ones.

Considering the adults, not as individuals, but as a group, and comparing the averages of the four series, we get the results as tabulated. Individual differences notwithstanding, we may say that, on the whole, the second set of trials, five days after the first, was distinctly slower; the third, two days after the second, not as slow as the preceding, whereas the fourth, six days after the third, was the best of all. Of course, these statements have no bearing on specific cases. The record as given is sufficiently detailed to show how the relative fixity in one individual is balanced, and even discounted by the relative instability of another, or vice versa.

INTRODUCTION OF ADDITIONAL STIMULI

The "tank experience" may be complicated by the introduction of additional factors, which, classified by their effects, may be called retarding and accelerating stimuli. Marked accelerations were produced by allowing the animals before reaching the water in the tank, to fall through a paper bag filled with water considerably warmer; also if, during their fall through the cylinder, the animals received an electric shock, the speed was increased. On the other hand, the addition of salt in small quantities to the water in the tank had, on the average, a depressing effect. The most marked, as well as constant effects, however, were gotten by the system of desired rewards. Animals that had been without food for twenty-four hours, were dropped into disagreeably cold water, and immediately on escaping, were wrapped in warm towels and given a nibble of cheese. All these factors helped to make escape from the tank better worth while than it had been in any of the preceding experiments. The results, based on two full-sized adults, and on eight young, less than a year old, are tabulated below. As heretofore, the adults are labelled with capitals, the young with small letters.

ANIMAL	FIRST HALF		SECOND HALF		IMPROVEMENT
	Record	Time	Record	Time	
	Trials	*Seconds*	*Trials*	*Seconds*	*Per Cent*
AA	6	7.45	6	3.71	51
BB	3	15.5	3	8.2	48
aa	6	10.8	6	3.2	69
bb	6	13.03	6	5.26	60
cc	6	9.8	6	7.3	26
dd	6	16.65	6	12.66	24
ee	6	13.3	6	7.2	46
ff	6	15.9	6	6.3	61
gg	6	18.8	6	3.2	83
hh	6	8.3	6	3.7	56

Not only are the adults too few, but the number of trials made is too small to allow of satisfactory comparison with the earlier records made under simpler conditions. Neither AA nor BB

improved as much as the adults in former experiments, but this may have been because they were unusually slow. If a conclusion may be hazarded, it is that the additional stimuli had no effect or perhaps a slightly depressing one, though this is uncertain.

The young, on the other hand, improved on the average 62.5 per cent, as compared with an earlier record of 53 per cent. Animals cc and dd have been eliminated from these calculations on the ground of over-excitedness. The results might have been expected. There was an unmistakable effect on the young, and in two cases the additional discomforts and rewards produced so much activity, that these particular records were vitiated by it.

THE SENSES AND THE HABIT

The relation between the senses and the acquisition of habits in the white rat has been so thoroughly worked over by Watson ('07) that I have performed only a few experiments, the results of which I shall present for two reasons, first because they are corroborative, in spite of the differences in method; secondly, because they show that the various senses can be eliminated without resorting to the extirpation, or destruction of the sense organs. Under the conditions of the experiments, hearing, smell, sight, and touch, either singly or in any combination, may conceivably furnish the sensual basis on which the habit rests.

HEARING

As the associations were formed while one of the four exits was open, it may have been that the movements of the animals were influenced by the differences in sound intensity or quality due to the unblocked passage. Such differences, if they exist, must be very small, for the closed openings are blocked simply by wire netting, but as rats are known to perceive minute sound differences, the point was well worth testing.

Associations were established in the usual way, after which

the exit to which the animal had been trained, was closed and one or several of the others were opened. In every case the individuals continued to go to the exit from which they had previously escaped. Of course this test could not be continued indefinitely, as failure to escape from the tank, resulted in new exploratory movements that in the end led the animals to one of the other openings. Frequent visits to the old corner are characteristic of these secondary trials.

In another experiment, the association was established amid general noise, so great just above the center of the tank, that probably there were no differences at any of the corners. When the animals, after a brief rest, were placed again in the tank, they were allowed to make their second series of trials in absolute silence. In all cases they swam to the right place with no more variation in time or in the complexity of the pathway than is found under ordinary conditions.

A third test was made by allowing the association to establish itself while a noise was made at one of the other opened escapes. These noises, purposely not loud enough to frighten the animals, but certainly marked enough to be audible, seemed to have no effect whatever.

SMELL

To determine the part played by the sense of smell, the exit left open was thoroughly perfumed with cheese. After the association was established, the tank was turned in such a way that the perfumed opening was diagonally opposite its original position, whereas another exit, unperfumed, occupied geographically the place previously held by the escape to which the animal had been trained. Variations of this plan, such as holding a piece of cheese, first in one corner, then in another, were also tried, but in each case the animals swam in the direction that led to the earlier escapes. So far as these tests of the importance of smell are concerned, they give only negative results.

SIGHT

The experiments on sight bear simply on such rays as we ourselves are able to perceive, and were performed in a carefully constructed and thoroughly efficient photographic dark room. From the subjective standpoint, the animals were allowed to swim in the dark, but whether what we call dark is in reality dark to a rat, is another question.

Two sets of animals were used, two adults, and two young ones less than a year old. Each animal was given four sets of six trials. The averages follow:

ANIMAL	DATE	TRIALS	AVERAGES
			Seconds
A	11/11	6	11.03
A	11/11	6	17.10
A	11/12	6	16.40
A	11/13	6	12.73
B	11/12	6	13.40
B	11/12	6	24.96
B	11/13	6	6.42
B	11/13	6	11.76
a	11/11	6	18.23
a	11/12	6	7.05
a	11/12	6	6.40
a	11/13	6	2.00
b	11/13	6	96.00
b	11/13	6	50.24
b	11/14	6	31.56
b	11/14	6	10.90

If the first two series of A are compared with the last two, it will be found that this animal remained practically constant in speed; if a similar comparison be made for B, we find that this individual increased its speed on the average by 9.28″; whereas the records of a and b show increases in efficiency in each of the last three sets. Undoubtedly, then, the tank problem can

be solved in the dark, and the solution, may with repetition, become a habit, but the evidence does not show that the sense of sight plays no part under other circumstances. Watson's negative evidence on this point seems very good, but the maze problem and the tank problem differ so much, that what is true in the solution of the one, is not of necessity true in the solution of the other.

TOUCH

Touch in the ordinary sense, is practically eliminated by the nature of the problem. The actual solution takes place in a medium in which the animals float. There are no solid bodies to be touched; no differences in homogenity in any direction, that might guide the individuals to the correct opening. The same thing applies to the temperature sense. It is possible, however, that touch and temperature, singly or together, affect the result, but if they do, it is not in the usual way, but as the initiating stimuli of other activities.

THE KINÆSTHETIC CHAIN

With hearing, smell, sight and touch, either eliminated, or shown to be not essential, the question of how the habit becomes established, naturally arises, Watson ('07) and others have presented a large body of evidence suggesting that the kinæsthetic sense may be the controlling factor in the behavior of the white rat. My own experiments seem to bear similar interpretation, but if the facts corroborated by the tank method are due to a kinæsthetic sense, it follows that this needs investigation.

Granted that a relation exists between the objective phenomena which we call a habit, and the inferred basal kinæsthesia, the inference that the relation is definite, seems just; for the habit itself is definite, and capable not only of exact measurement, but also of modification. It follows that in the emergence of a habit, the internal basis also is modified, for the habit itself is modified behavior. In other words, kinæsthesia is easier to understand if

thought of as a sequence of states which will be repeated, provided the physiological state of the animal remains favorable, whenever the stimuli that started it in the first place recur.

It is here that any sensations associated with the beginning of a solution may come into play. Unfortunately, I have no results to offer on this subject at present, but the standpoint itself may not be without value. If kinæsthesia is indeed a special element in the sum total of internal conditions, is in fact a chain, based on the physiological, and expressed objectively in the habit, the answer to a difficult problem may be a little easier to find than heretofore.

SUMMARY AND CONCLUSIONS

My purposes in presenting these results have been to show the adequacy of the tank as an instrument for studying animal behavior; to show that our usual methods leave out of consideration a large range of interesting activities; and finally to demonstrate that behavior, limited to brief periods of very intense exertion under stress, may lead to the formation of habits.

Incidentally, the results seem to corroborate, as far as they go, some of the conclusions set forth in Watson's splendid work, and wherever at variance, are so probably from the differences between the problem of the maze, and the problem of the tank. In certain ways, the latter seems well suited for studying the "direction sense" or whatever it is that in the absence of sight, hearing, smell, and touch, enables the animals, not only to solve the problem, but to improve in efficiency. If kinæsthesia plays an important role, if indeed it is by this means that the animals sense direction, and if furthermore, it is a sequence of states, dependent on the physiological condition of the animal, and on certain initiating stimuli, then by variations in the last two categories, one should get changes in kinæsthesia, which in turn would be registered by corresponding differences in the objective habit.

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Zoölogical Laboratory, University of Michigan.
January 13th, 1910.

ON THE MEDIAN ANTERIOR CEREBRAL ARTERY AS FOUND AMONG THE INSANE

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From the Government Hospital for the Insane, Washington, D. C.

WITH SIX FIGURES

The subject of anomalies of the cerebral arteries among the insane has received considerable attention in the past, especially noteworthy being the article by Bullen in the Journal of Mental Science, volume 36, 1890,[1] but unfortunately comparative statistics are meagre. In 1907 I made a study of the conditions of development of the encephalic arteries in 220 consecutive cases of mental diseases, making a comparison with the studies of Windle[2] in 200 cases of those presumably sane. The results of this comparison seemed to show a decided predominance, in general, of anomalous conditions among the mentally diseased.[3]

In the study above mentioned, being chiefly concerned with the anomalies of the circle of Willis, sufficiently careful studies of other arteries were not made, and as a result, the anomalous vessel which forms the subject of this paper was not as frequently found as subsequent observations show is probably the fact. This artery is found of course among the sane as well as the insane. My studies, however, are based upon the examination of 400 consecutive cases of mental disease examined with special reference to this vessel. In all of the text books of anatomy at my com-

[1] BULLEN. Post mortem Examination of the Brain, etc. *Journal of Mental Science*, vol. 36, 1890.

[2] WINDLE. On the Circle of Willis. *Reports of the British Medical Association*, for 1887. *New York Medical Journal*, vol. 2, 1888, and *Journal of Anatomy and Psysiology*, 1887–1888.

[3] BLACKBURN. Anomalies of the Encephalic Arteries among the Insane. *Journal of Comparative Neurology and Psychology*, vol. 17, no. 6, 1907.

mand I have either found no reference to the artery at all, or a mere mention of it, as by Cunningham, as a third anterior cerebral artery sometimes present, or by Quain, as found in 4.5 per cent of cases.[4]

In an article in the *Journal of Nervous and Mental Disease*, volume 12, no. 3, July, 1885, under the heading, "On a seldom described Artery (Arteria Termatica) etc," Professor Wilder describes the artery and gives it the name of *Arteria Termatica*, from the place of location and chief distribution in his cases. Professor Wilder thus describes the vessel which he found in a large percentage of human brains examined:

> It usually soon divides into a right and left portion which supply respectively the cinerea forming the surface of the triangular area ventrad of the rostrum on either side, and then extend around the genu to the dorsal aspect of the callosum.

In another place in the same paper, he describes its origin, as most frequently from the place of junction of the precerebrals, the pre-communicant being absent, an important observation in connection with the present subject. In some respects most of my cases of this artery have varied somewhat from Wilder's description, being as a rule larger, and following more closely the description of the median anterior cerebral artery as described by Windle, who says,

> It passes along the longitudinal fissure for two-thirds of the length of the callosum, and divides into two branches, supplying the opposite surfaces of the hemispheres.

Windle found this artery present in 9 of the 200 cases examined, and as his description of it is unqualified, it may be concluded that most of his cases were of the larger-sized vessels, perhaps more properly named the median anterior cerebral artery, though I have no doubt that Wilder, Windle and I are describing the same vessel.

[4] Quain's Anatomy, 1892. Other text books of Anatomy.

In a typical median anterior cerebral artery, the description is as follows:

The vessel arises from the anterior communicating artery, usually near the middle; curves upward, and in close proximity to the lamina terminalis, around the genu of the callosum, lying close to this body; and at about the middle or at the junction of the anterior two-thirds with the posterior third of the callosum it divides into two nearly equal branches which are distributed to the paracentral lobules and the quadrate lobules of the opposite hemispheres. In the course of the main trunk and the principal branches of the vessel, small and unimportant branches are given off to the lamina terminalis, the callosum, and to the adjoining gyri, but these ultimate branches have not been worked out, and are perhaps relatively unimportant. A large majority of these aberrant vessels have this origin and distribution, though occasionally the origin may be nearer to one anterior cerebral artery than the other. It may even seem to originate from one of these vessels near to the junction with the communicans, or what is still more important for our study, it not infrequently arises from the end of the junction of the two anterior cerebrals when these are fused and the anterior communicating artery as such is absent.

Differences in the size and distribution of the main branches are occasionally met with; sometimes one branch may be wanting, and the vessel is then distributed to one side only. In the small or undeveloped vessels as those described by Wilder, the branches are all small and irregular, and are sent mainly to the lamina terminalis, the rostrum callosi, and the gyri in the immediate vicinity.[5]

In an article by Grünbaum and Sherrington in *Brain*, 1902,

[5] I find that on carefully removing the basal arteries and floating them out in water, often a long, slender, arteria termatica is unexpectedly revealed. This leads me to suspect that arteries of this type are quite frequently present but are undiscovered; probably the percentage of median anterior cerebral arteries might be nearly doubled if careful search were thus made. Whether these arteries are vestigial or reversionary, it is certain that in the majority of instances no such vessel arises from the anterior communicating artery, and that no corresponding vessel is mentioned among the branches of the artery by most anatomists.

"Note on the arterial supply of the Brain in Anthropoid Apes," these observers state:

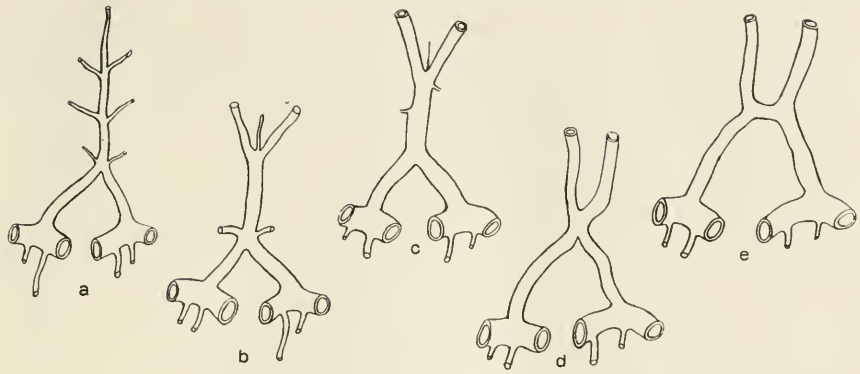
> Among features of human character pertaining to the anthropoid apes, we find one not hitherto recorded, namely the existence in the cerebral arterial supply of a *Circulus Willisii* resembling that of man. In these highest apes, as in man, an anterior communicating artery quite frequently completes the *circulus* in front;

and, quoting Parsons, they add:

> In all of the lower mammals which I have examined, including the Platyrrhine monkeys, *Cercopithecus*, etc., I find no anterior communicating artery.

In all of the lower forms of mammals instead of the pair of anterior cerebral arteries, there is but a single azygos vessel, which in its course forward gives off branches to the right and left hemispheres respectively. These observers examined six brains of chimpanzees, and one orang-outang. Of the six chimpanzees, in five the circulus had the human type. In one of these two anterior cerebral trunks were united or fused into a common trunk about four mm. in length. In the orang-outang also the circulus was completed by an anterior communicating artery, and in one of the six chimpanzees there was a single azygos anterior cerebral artery as in the lower mammalian forms. This work, which is in accord with the writer's limited experience, shows that the existence of an azygos vessel in place of the anterior communicating artery and the two anterior cerebrals is the rule in the lower mammals, and that the form found in the higher anthropoid apes and in man is a phylogenous development while it also suggests that in view of the frequency of anomalous forms in the anterior cerebrals, that this phylogeny is as yet unstable.

In these arteries, anomalies of development are by all odds more frequent than in any other sets of vessels. Windle in 200 cases found 8 of fusion, 9 median anterior cerebral arteries, and a large number of anomalies of the anterior communicating artery; in my own 220 cases, fusion of the vessels was found in 7 cases, in



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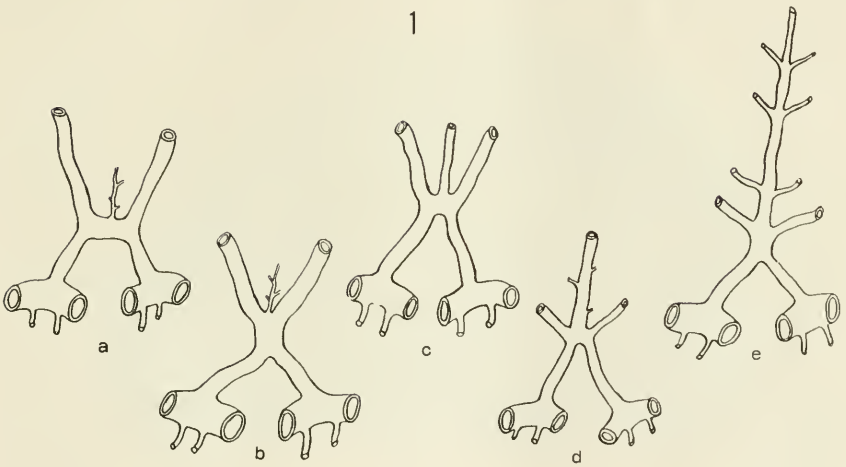

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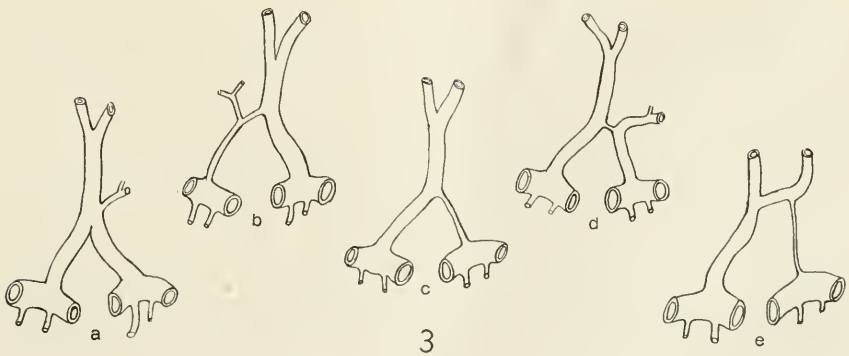

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FIG. 1. a—e. Developmental types of the anterior system of cerebral arteries.
FIG. 2. a—e. Reversionary types of the anterior cerebral arteries.
FIG. 3. a—e. Types of anomalies of the anterior cerebral arteries.

10 the right artery was very small, and the opposite carotid artery sent the blood supply to the two hemispheres through an enlarged anterior communicating artery, and in 6 cases the left artery was the smaller, and the main blood supply came from the right carotid system; in a large percentage of cases, anomalies of the communicans were found. It is possible that in many of the apparent anomalies of the anterior cerebral arteries with disproportion in size of the trunks beyond the anterior communicating artery, the larger vessel may be a partial reversion to the primitive type; that is, a median anterior cerebral unusual only in place of origin. In fact, it is not at all uncommon for the larger of the two trunks to send branches to the mesial surfaces of both hemispheres.

It may then be granted that the lower types are being developed in the phylogeny of the races, and such being the case, reversion to the primitive type may now and then be expected. It has seemed to the writer that the development and reversions might be shown graphically in the accompanying drawings, nearly all of which are drawn from actual specimens. In Fig. 1, *a* represents the primitive form as met with in the lower mammals; *b* shows the fusion of the anterior cerebrals with the remnant of the azygos vessel at the end of the junction; *c* a later stage of the development, with the remnant of the azygos vessel; *d* shows the shortening of the place of junction, and *e* shows a fully developed anterior communicating artery. Fig. 2 shows the occasional reversions met with; *a* gives the type of arteria termatica of Wilder; *b* gives the type of termatic artery which arises from the end of the short fusion of the two vessels; *c* shows the most common form of median anterior cerebral artery; *d*, that in which the anomalous artery is the largest of the three, and *e*, the almost complete reversion to the lower mammalian type; *e*, however, has not actually been seen by the writer, while *d* is common.[6]

It is suggested by these forms that in the higher phylogenetic development of the brain, the greater size of the frontal lobes has

Quain says: "The two arteries have also been seen united in a single trunk, which runs in the longitudinal fissure, giving off branches to both hemispheres." The writer has recently found one of these vessels, hence the series may be regarded as complete.

necessitated a development of two anterior lateral branches of the azygos vessel into the two lateral anterior cerebral arteries, and that with this has progressively gone on a shortening of the fusion of the two vessels and an atrophy of the azygos terminal, until finally we find the human type of this vessel, the anterior communicating artery, and the two lateral cerebrals. The fact that we occasionally meet with a median vessel, with fusion of

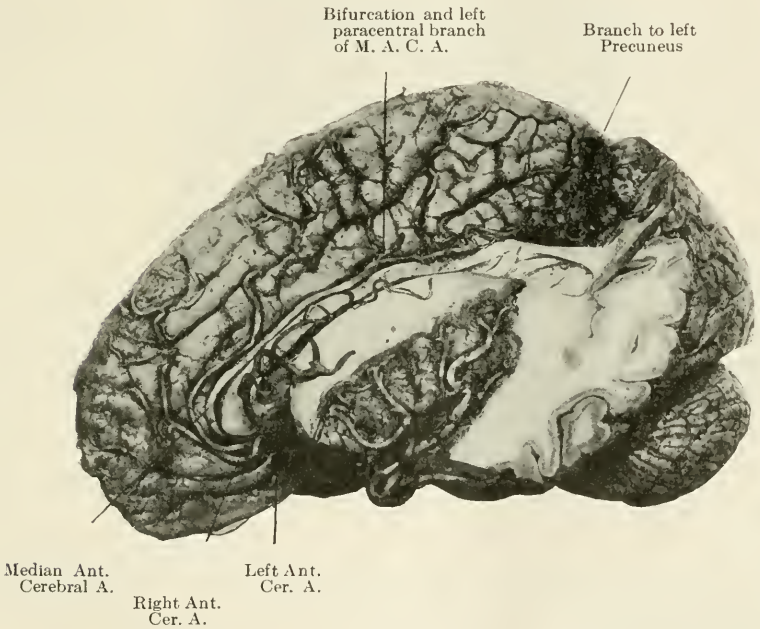

FIG. 4. Photograph of a dissection showing the two lateral anterior cerebral arteries and a large median anterior cerebral artery with a typical distribution.

the two anterior cerebrals, and other anomalies of this set of vessels, I think may be accounted for on the principle of reversions and anomalies so frequently found among the insane.

The median anterior cerebral artery has been found in all forms of mental diseases, possibly a little more frequently in forms of dementia. In 400 cases of mental disease, 42 instances of the abnormal vessel were found; 11 were in senile dementia, 7 in chronic

dementia, 5 in epileptic dementia, 5 in arteriosclerotic dementia, 4 in paresis, 3 in chronic melancholia, 2 in dementia praecox, and 1 case each in manic-depressive insanity, organic dementia, acute delirious mania,acute mania, and idiocy. Twenty-five of these cases were in white males, 11 in colored males, 4 in colored females and 2 in white females. The disproportion between the cases examined of each color and sex makes this of relatively little value.

I do not see that there can be any special relationship between the mental disease *per se* and the presence of this anomalous vessel, but on the supervention of arterial diseases some very interesting cerebral conditions might be caused. In most instances, cerebral arterial anomalies are compensated for before the develop-

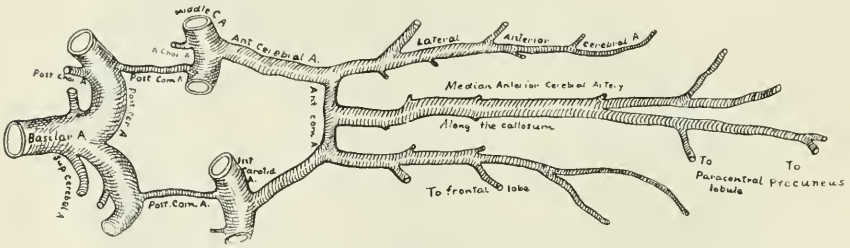

FIG. 5. Diagram of the Circle of Willis with a typical median anterior cerebral artery.

ment of the psychosis, the anomaly being then only indicative of ill development.

In these days of bold operations upon the vessels and even on structures at the base of the brain, it might be well for the surgeon to bear in mind the existence of these anomalies. In fact the main object of the paper of Grünbaum and Sherrington, quoted from above, was to call attention to the surgical relations of the inequalities of cross anastomosis of the circulation in anomalies of this anterior arterial system. Illustrative of these dangers may be mentioned one of my own cases in which thrombosis of the intracranial portion of the carotid artery resulted in death of the

whole left hemisphere on account of small size and sclerotic obstruction of the anterior communicating artery and the left posterior cerebral artery.

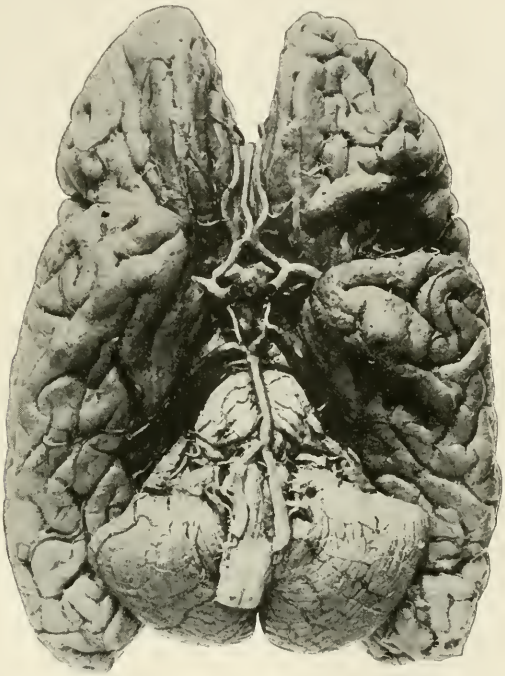

FIG. 6. Photograph of the base of the brain showing the origin of a large median anterior cerebral artery.

The conclusions which I think we may reach with certainty are as follows:

1. There is a gradual development of the lower mammalian form of azygos anterior cerebral artery into the perfect *Circulus Willisii* of the human type.

2. The frequent variations of this system of vessels suggest instability of ontogeny, and of phylogeny.

3. The anomalies of these arteries together with the presence

of the median anterior cerebral artery are frequent among the insane.

4. That in keeping with other anomalies of the brain among the insane it is probable that many of these variations are in the direction of reversion to the primitive type, and that it is reasonable that the frequently found arteria termatica is one of these reversions or survivals such as are not uncommon in other organs.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. No. 208.

DEGENERATION IN THE GANGLION CELLS OF THE CRAYFISH CAMBARUS BARTONII GIR.

HANSFORD MacCURDY

WITH NINE FIGURES

INTRODUCTION

Much of our knowledge of the changes occurring in the central roots of nerves and their ganglia after the nerve trunks have been severed has accumulated during the last two decades. In the earlier observations and experiments, attention was directed chiefly to the nerve roots and their related ganglia. From various causes, including the complex character of the nerve centers, the earlier investigations did not include the ganglion cells. Only comparatively recently has attention been directed specially to the ganglion cells and the changes occurring in them.

It was suggested to me by Professor G. H. Parker that the large nerve cells of the abdominal ganglia of the eastern crayfish, Cambarus bartonii, would afford favorable material for the study of the changes in the ganglion cells after their nerve fibers had been severed. Inasmuch as the investigations hitherto reported have been on the nerve cells of vertebrates, the additional purpose of extending our knowledge to an invertebrated animal would also be served.

As it is well known, Waller held that only those parts of the nerve fibers degenerate which are separated from their nerve centers. For many years this view prevailed. In time, however, from observations made in cases where limbs had been amputated which revealed an altered condition of the nerve roots and their ganglia, doubts arose as to whether Waller was correct in limiting the changes to the peripheral parts of the affected nerves. The

observations of Friedländer und Krause ('86) showed that alterations in the roots of the severed nerve were considerable. In long-standing cases of amputations, these authors reported alterations of medullated fibers, and a reduction in the size of the bundles of fibers. These observations led to experiments on animals for more direct evidence on the questions involved. Homén ('90) performed experiments by cutting nerves in a number of dogs and found that the cells of the related ganglia were much reduced in size. He attributed this reduction to atrophy of the ganglion cells and their nerve fibers. Krause ('87) expressed the opinion that true retrograde degeneration is identical with Wallerian degeneration. Marinesco ('92) found that on cutting the spinal nerves marked changes occurred in the dorso-lateral group of cells in the spinal cord, and stated that the number of these cells was much less in animals operated upon than in the case of normal animals. He also claimed that the remaining cells showed more or less atrophy. In experiments in which the ganglion nodosum was concerned, he (Marinesco, '98) described a period of restoration after the period of depression.

Fleming ('97), operating on dogs and rabbits, measured the nuclei of the affected nerve cells and at the end of 12 days found them to be smaller than the nuclei of normal nerve cells. At the end of eighteen weeks, he found still greater differences in the size of the affected and normal nuclei and the noted some atrophy and the disappearance of some cells. He also described differences in the size, position, and arrangement of the chromatic elements in the cells. In experiments on rabbits, Van Gehuchten ('97) found the majority of the ganglion cells of the ganglion nodosum degenerated. Similar results were obtained from the dog by Kosaka und Yagita ('05). Köster ('03) gave an account of a series of experiments on cats, dogs and rabbits in which the sciatic nerve was cut near its exit from the vertebral canal. He described differences in the tigroid bodies of the spinal ganglion cells. In some of the cells a partial restoration occurred, while in others complete degeneration was found. A modification of the tigroid substance of the cell protoplasm was found to take place four to six days after the operation, while degeneration occurred only

after a much longer interval of time. Kleist ('04) used in his experiments half-grown cats and rabbits and found degeneration of the spinal ganglion cells of the upper cervical and lower thoracic nerves which had been severed. He further described some of the cells which did not degenerate as having undergone distinct atrophy, while others, he concluded, returned to a normal condition, since he found more cells at the end of four months than were seen at the end of ten days. Van Gehuchten ('03) found degeneration taking place centrally and declared it to be a true degeneration, *i.e.*, the same as that which takes place peripherally. He also pointed out the importance of this process in tracing the course of nerves.

Ranson ('06) operated on white rats by cutting the second cervical nerve and found simple atrophy and true degeneration in the ventral and dorsal roots, the spinal nerve ganglia, and the spinal cord. An apparently variable number of ventral-horn cells disappear as a result of degeneration, likewise a considerable and constant number of spinal ganglion cells. To avoid septic infection of the parts studied, Ranson used methods which should satisfy every requirement.

It is seen that in a majority of cases changes are said to occur in the central parts of the nervous system after nerves have been severed. These changes consist in a reduction in the size of the fibers and the ganglia, and, in fewer cases, in the disappearance of the nerve fibers and nerve cells. Perhaps the most convincing evidence of the disappearance of the ganglion cells is that found in the results of Ranson in the disappearance of one-half of the nerve cells in the spinal ganglion after cutting the second cervical nerve in the white rat.

EXPERIMENTAL ON THE FIFTH ABDOMINAL GANGLION OF THE CRAYFISH

Retzius ('90) long ago described the structure of the abdominal ganglia of Astacus fluviatalis. His work renders unnecessary a complete description of these ganglia in Cambarus, the form used

in these experiments. The fifth abdominal ganglion was selected as the most suitable for study, because its position rendered it accessible for operative purposes and its structure appeared reasonably simple as to the number and distribution of the large ganglion cells. In this ganglion, in its normal condition, are found a few relatively large ganglion cells whose positions are fairly constant and whose identity can be determined with certainty, at least in most cases. Of these large cells a few lie well forward in the ganglion, near its ventro-lateral surfaces, forming a group on each side of the ventral line. In each of these groups there are a few easily recognized cells larger than the others. These lie in the anterior third of the ganglion. Immediately posterior to each anterior group of cells, though not distinctly separated from them, is another group of cells, which lies for the most part beneath the roots of the large lateral nerves. In this region are three or four nerve cells which are usually the largest found anywhere in the ganglion. A transverse section passing through the roots of the lateral nerves usually cuts through one or more of these large ganglion cells. In the section, these cells lie near the ventral surface of the ganglion and to the right or left. It is thus seen that the identification of the particular cells is reasonably certain. Posterior to the group of cells just described, and closely connected with it by the smaller cells, lie the remaining large ganglion cells which form the posterior part of the ganglion. As in the other parts of the ganglion, the cells of the posterior group vary in diameter, and individual cells may be identified by their position as well as by their size. It is to the large ganglion cells in the positions which have just been pointed out that attention is particularly directed later.

The smaller cells were less suited to the requirements of the experiments than those just described because of the difficulty with which their finer structure could be determined. Of the other abdominal ganglia only the fourth and sixth need be mentioned. While the fourth ganglion was not studied as fully as the fifth, it was clear that the large cells were arranged in it in much the same way as in the fifth, and that the disappearance of any cell or cells from this ganglion could be easily recognized. The sixth

ganglion is larger than the other two and contains a greater number of ganglion cells, whose arrangement was different from that in the other ganglia. No attempt was made to determine any particular arrangement of the cells in this ganglion. On this ganglion the general effects of the operation were observed as well as the degeneration and disappearance of its cells without respect to their particular position.

In all these ganglia, each large ganglion cell has a single nerve fiber proceeding from it, which may be traced in favorable sections far enough to determine its course some distance through the ganglion. Retzius ('90) has shown that the fibers from some of these cells pass into the connectives and from others into the lateral nerves. These fibers are non-medullated, having only the sheath of Schwann. The fibers also show very characteristic nerve fibrillæ. These fibrillæ are best seen in the large fibers when special methods of fixation are employed. They may also be seen very distinctly in the axis-cylinder within the ganglion cells, where they extend partly around the cell nucleus, though separated from it by a certain amount of the protoplasm of the cell. It is evident from what has been said that these large ganglion cells with their fibers extending outward in the manner described, afford relatively simple conditions for experiments, in which the effects on the ganglion cells following the cutting of their fibers could be readily observed.

It was not known how well the animals could endure the injuries incident to the necessary operations, such as the effects due to shock and the interference with the ventral blood sinus. Throughout the series of experiments, however, little difficulty was experienced from either of these sources. There was but little loss of blood, shock effects soon passed away, and the wounds healed with greater promptness than was anticipated. Aside from the loss of movement of the fifth abdominal segment and those posterior to it, the animals operated upon differed from normal individuals only in that they were slightly less active.

In operating, an incision was made with a sharp lance through the integument well toward the right side of the ventral surface of the abdomen between the fourth and fifth sternites. By insert-

ing the lance, or a fine-pointed pair of scissors, the connective was severed about midway between the fourth and the fifth ganglion. Through the same incision the instrument was passed backwards in a line nearly parallel to the long axis of the body, severing the lateral nerves on the right side of the fifth ganglion. Another incision similar to the first was made to the left of the median line between the fifth and sixth sternites and through it the connective between the fifth and sixth ganglion and the left lateral nerves of the fifth ganglion were severed. A thin coat of celloidin was then applied to the wounds. Thus the connectives, both in front of and behind the fifth ganglion, and the lateral nerves on each side of it were cut with the least possible amount of injury to the tissues, and the ganglion was thus isolated as regards its nerve connections. The animals were then numbered for purposes of identification, placed in an aquarium, and propery cared for until they were taken for study. Only those animlals in which the wounds healed readily and which showed no evidence of infection, etc., were selected for final preparation and study.

At desired intervals after the operation, individual animals were killed, and the abdominal ganglia from the third to the sixth inclusive were removed together by cutting along the ventral surface through the integument and sternites on each side of the median line, and carefully removing the ventral wall of the body with the nerves and ganglia *in situ*. The entire piece was kept straight by attaching it to a glass rod and in this position it was immersed at once in the killing fluid. Before clearing, the nerve elements were carefully removed from their natural position on the body wall and transferred together through absolute alcohol and xylol and imbedded in paraffin.

Since the cells of the isolated and the normal ganglia were finally to be carefully compared, it was necessary to give them, as nearly as possible, the same treatment. To secure this equality, one normal individual and one individual which had been operated on, were killed and prepared together in the manner described and given parallel treatment throughout.

Two methods of treatment were used. One series was prepared according to the Nissl method for staining the tigroid substance,

and another was treated with vom Rath's picro-aceto-platino-osmic fluid.

In using the Nissl method, the ganglia were put for forty-eight hours in 95% alcohol, then dehydrated, cleared in xylol, imbedded in paraffin, cut in sections 10μ. thick, and stained in toluidine blue. In order to secure perfectly parallel treatment of the cells in the staining, washing, and subsequent dehydration, serial sections of the isolated ganglion of a normal individual and of an individual operated upon were placed in alternate rows on the same slide. Preparations were made in this way from materials killed at intervals of from two to five days covering a period in all of thirty-three days after the operation.

The large nerve cells of the normal ganglia prepared by the Nissl method presented a characteristic appearance (fig. 1). Fibrillæ in the axis cylinder were usually visible, though not all the cells revealed them. That they were not always seen, is believed to be due to this method, which, as is well known, is not a wholly satisfactory one for demonstrating fibrillæ. The Nissl "flakes" were present, though somewhat less distinct than in the ganglion cells of vertebrated animals. In some cases there appeared, more or less distinctly, small centers, which stained deeply and from which radiated irregularly threads of protoplasm. These threads or strands connected with other similar centers or faded out in the surrounding cytoplasm. These small centers or granules with the network of radiating threads are confined almost wholly to regions adjoining the axis-cylinder area. The nucleus exhibited a full rounded form and possessed a reticular structure with a very distinct and deeply stained nucleolus. Occasionally two nucleoli were found in one nucleus. Sometimes a slight shrinking of the cytoplasm next the nucleus was observed.

With the state of the normal ganglion cells, are to be contrasted the conditions found in the corresponding ganglion cells from a fifth ganglion (fig. 2) which had been nervously isolated about twelve days, but otherwise had received identical treatment. The cells from the isolated ganglia showed distinct alterations. The nerve fibrillæ in the axis-cylinder area had disappeared and the Nissl flakes had become finely granular or had disappeared.

The reticulate structure was lost, and the cytoplasm had shrivelled away from the outer parts of the cell. The nucleus also shared in these changes. The nuclear membrane had suffered a complete collapse, while the nuclear network had disappeared. The nucleolus apparently was more resistant than the other parts, and only after longer periods than that shown in figure 2 did it finally fail to stain and ultimately disappeared.

Degeneration may be practically complete at the end of two weeks, or it may be prolonged through a much greater period. In addition to the changes in the large ganglion cells, many of the smaller cells undergo corresponding changes and disappear. The ganglion is much reduced in size because of these changes. Preparations made three to five days after the operation usually showed but little change. After seven to fourteen days the changes were quite evident. In some cases cells had lost their distinguishable structures eighteen days after the nerves had been severed, while other cells in the same ganglion were apparently only slightly affected. The disappearance of individual cells in the midst of cells which still remained apparently little affected is regarded as strong evidence of true degeneration.

On examining the results obtained in the first series of experiments, it became evident that, although degeneration could be demonstrated, the Nissl method had revealed only a part of the changes which had occurred within the cells. Some method which would show the changes in the finer structure of the cells and one which would serve as a check on the previous experiments was, therefore, sought. Since the picro-aceto-platino-osmic fluid of vom Rath is known to demonstrate admirably the finer structures of these ganglion cells in their normal condition, it was thought that this treatment might also show the changes occurring in connection with the loss of function and consequent degeneration. Accordingly the ganglia were removed, in the manner described for the Nissl method, from an individual in which the fifth ganglion had been isolated twenty-nine days, and were immersed in vom Rath's fluid for a period of forty-eight hours, washed in water, dehydrated, cleared in xylol, imbedded in paraffine and cut into sections 10μ thick. Examination of the material revealed nerve

cells in various stages of disintegration in the fourth, fifth and sixth ganglia. This unmistakably demonstrated the usefulness of this method for detecting degenerating cells. In the fourth ganglion, certain large nerve cells were almost wholly disintegrated, while adjoining nerve cells were apparently normal. This is considered significant, since this ganglion had only the connective between it and the fifth severed. A similar condition was found in the sixth ganglion, which had only its anterior nerve connections severed. The fifth ganglion showed more extensive changes, which will be referred to later.

By this method preparations were made of material *in situ* at intervals of three or four days over a period of thirty-eight days from the time of operation. Corresponding normal ganglia were given treatment at the same time and mounted in parallel series with the isolated ganglia in a majority of the experiments. In later experiments parallel series were deemed unnecessary because of uniformity in results.

Were it not needed for comparison, it would be unnecessary to give even a brief description of the normal ganglion cell prepared according to the vom Rath method. The action of the fluid on the nerve cells is such that the various cell structures are well differentiated in black and white. The fibrillæ (fig. 3) are very clearly seen in the axis cylinder within the cell as very fine dark lines. They are frequently also seen in the protoplasm of the cell surrounding the nucleus. Numerous flake-like bodies, apparently having some relation to the Nissl bodies, lie in the cytoplasm, arranged concentrically around the nucleus. This concentric arrangement of parts around the nucleus is a condition which will be referred to in a future paragraph. The nucleus shows the characteristic nuclear membrane, network, and nucleolus.

In ganglia which had been isolated five to seven days, some of the large ganglion cells had begun to show alterations in their structure. The large cells in the posterior part of the ganglion were among the first to become affected. Some of the smaller cells in this part showed on-coming changes somewhat earlier. In the large cells the fibrillæ (fig. 4) became somewhat nodular and tortuous in their course. The limits of the axis-cylinder area in

EXPLANATION OF FIGURES

All figures were drawn with the aid of the camera lucida and are magnified 375 diameters. Figures 1 and 2 are from preparations made by the Nissl method; the others are from preparations made by the vom Rath method; all are from the fifth abdominal ganglion of Cambarus bartonii.

1. Large ganglion cell, normal; Nissl method.

2. Large ganglion cell from ganglion isolated twelve days; nucleus collapsing, axis-cylinder area reduced, fibrillæ indistinct.

3. Large ganglion cell showing normal condition of axis cylinder, fibrillæ, nucleus, and "flakes;" vom Rath method.

4. Large ganglion cell from ganglion isolated twelve days; tortuous and nodular fibrillæ. Compare with figs. 2 and 3.

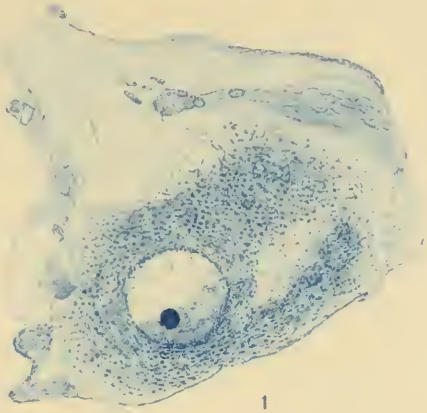
5. Ganglion cell from ganglion isolated fifteen days; nucleus and axis cylinder reduced, fibrillæ breaking up, cytoplasm granular.

6. Ganglion cell from ganglion isolated twenty-six days; nucleus collapsed, fibrillae absent, cytoplasm granular.

7. Ganglion cell from ganglia isolated twenty-eight days; nucleus and nucleolus disappearing, cytoplasm granular.

8. Ganglion cell from ganglion isolated thirty days; nucleus and nucleolus disintegrating (seen in only a few cells), cytoplasm faintly granular.

9. Ganglion cell from ganglion isolated twenty-nine days; nucleolus has disappeared; cell-contents faintly granular and apparently very fluid.



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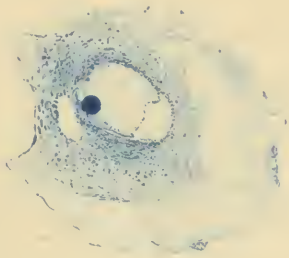

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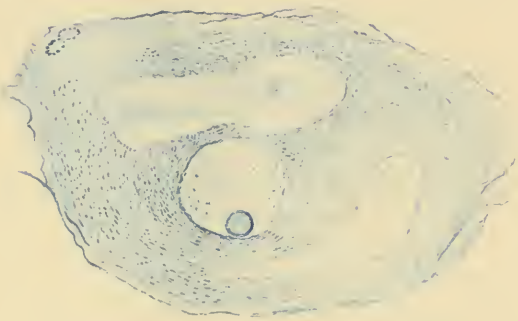

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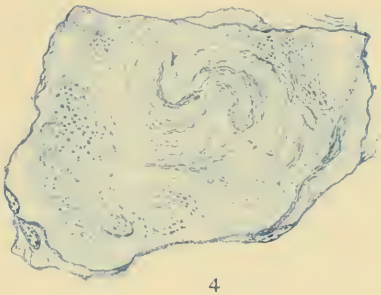

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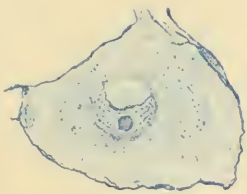

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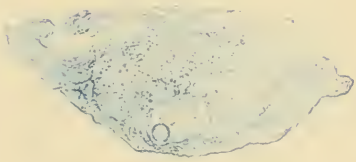

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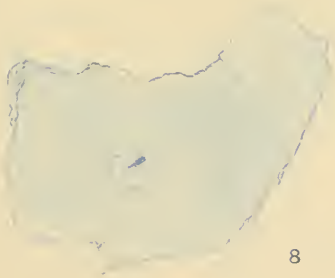

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the cells were less clearly defined, and the normal concentric arrangement of the parts about the nucleus was more or less disturbed. Usually in twelve to fourteen days after the operation these changes became very evident. At this stage the fibrillæ had become more nodular and tortuous in appearance and the axis-cylinder area was usually lost in the increased disturbance in the arrangement of the cytoplasm of the cells. In addition to these alterations in the organization, a marked change was seen in the results of the action of the vom Rath fluid on the various parts of the cells, in that they did not become black, as in the case of normal cells, but assumed a somewhat yellowish color, instead. This chromatolysis is one of the most characteristic alterations observed in these cells and indicates chemical changes in their constitution. This appeared early and increased as degeneration advanced. This change is undoubtedly the chromatolysis demonstrated by the Nissl method and observed in the spinal-ganglion cells of dogs by Lugaro ('87), and in cats, dogs and rabbits by Köster ('03). In eighteen to twenty days the fibrillæ and axis cylinder usually had entirely disappeared (figs. 5, 6), and the concentric arrangement about the nucleus had entirely broken up. The flake-like bodies in the cytoplasm had given place to fine granules. The nucleus (fig. 6) was usually collapsed and the nucleolus had also undergone chromatolysis. The later stages (fig. 7), which were usually found at eighteen to thirty days after the operation, were marked by the gradual loss of the various parts of the cell. The fine granules visible earlier in the cytoplasm disappeared, the nucleoplasm shrank, and the nuclear membrane disintegrated. The nucleolus remained slightly longer than the other organs of the cell and was seen sometimes to disintegrate (fig. 8) and sometimes to grow fainter and fainter until it became invisible (fig. 9). The order of these events rarely varied.

The number of cells in the fifth ganglia which were affected varied in different cases. This depended in part on the time that had elapsed since the operation, and no doubt in part on slightly varying conditions of the state of the animal. In no case did all the ganglion cells in any ganglion disappear, and in no case did degeneration fail to occur in a considerable number of cells within

the limits of the experiments. Three individuals in which the fifth ganglion had been isolated were not killed until ninety days after the operation. These three presented a condition of the ganglia and ganglion cells very similar to that found in ganglia at the end of thirty-nine days. The parts of the ganglion which had undergone degeneration were somewhat more clearly distinguished from the remaining portion. There was some evidence that partial regeneration had occurred in these ganglia, but this was not conclusive.

THE FOURTH AND THE SIXTH ABDOMINAL GANGLIA

In these experiments, as already stated, the connective between the fourth and fifth abdominal ganglia was severed a few millimeters posterior to the fourth ganglion; some cells in this ganglion might, therefore, be expected to degenerate. In all cases the fourth ganglion was prepared in the same manner as the fifth and this included the posterior end of the connective. An examination of these ganglia revealed degenerating cells in their anterior parts on both right and left sides, and a few cells in their middle portions on each side near the ventral surface. These were most clearly seen twenty to thirty days after the connective had been severed. In these cases the nerve cells in the posterior part of the ganglion were but little influenced. In certain cases of longer standing some of these posterior cells suffered partial degeneration.

The sixth abdominal ganglion was prepared in the same way as the fourth and fifth. Degeneration of a considerable number of nerve cells in the anterior parts of this ganglion was found in all cases twenty or more days after the connective had been severed. A smaller number of nerve cells in a similar condition of disintegration were found near the ventral surface in the sections through the middle and posterior portions of the ganglion. The beginning of degeneration in this ganglion was found in preparations made twelve to fourteen days after the operation.

The degenerating ends of the connectives were included in the preparations and mounted in series with the related ganglion.

The nerves showed a more advanced state of degeneration at and near the cut surfaces than was found at points more remote from these surfaces.

DISCUSSION

It has been found in the experiments described in this paper that the ganglion cells in the fourth, fifth and sixth abdominal ganglia of Cambarus bartonii undergo distinct structural changes when the connectives and lateral nerves are severed. A larger number of cells is affected in the fifth ganglion than in the others; this ganglion, from the nature of the operation, had more of its nerve fibers cut than either of the other two. That these changes were due to the cutting of the nerve is beyond reasonable doubt, since they were found only in those ganglia which had their connectives severed, and were found in every such case. That the changes are those connected with true degeneration finds support in the fact that they resemble in their essential points the histological changes in the corresponding parts of the nerve fibers and cells of vertebrated animals as described by Mönkeberg und Bethe ('99), Kleist ('04), and others. In all cases chromatolysis is reported to have occurred soon after the nerves were severed, which indicates important chemical changes in the nerve cells. Following this were characteristic alterations of the nerve fibrillæ and other structures of the cell, resulting finally in the destruction of the cell itself. Kleist ('04) stated that two to six days after the sciatic nerve in dogs, cats and rabbits had been severed, alterations occurred in the tigroid bodies of the spinal ganglion cells. In ten days vacuolar degeneration and shrinking of the cells had occurred. It has been shown in this paper that alterations in the tigroid bodies of the ganglion cells of Cambarus were found five to seven days after the connectives had been cut, and that in ten to twelve days the fibrillæ were distinctly altered and a shrinking of the cytoplasm and nucleus had occurred.

In regard to the time required for complete degeneration to take place, experimental results vary widely. Fleming ('97) observed disintegrating nerve cells in six weeks, but many more in eighteen weeks. Köster ('03) found that only relatively few cells had de-

generated in three months, but a large number had disappeared after nine months. Ranson ('06) observed no further change after two months, and for this reason concluded that degeneration was not progressive. In the present series of experiments some cells had lost all distinguishable structures in twenty-one days, and a greater number was found in this condition twenty-eight to thirty-nine days after the injury. It is, therefore, reasonable to believe that degeneration takes place slowly in some cases and more rapidly in others. These differences are best explained by the differences in the animals used and in the nature and the conditions of the experiments.

The results of this series of experiments furnish some additional support to the view that the continued life of the neurone depends upon the performance of the normal functions of all its parts.

SUMMARY

1. Observable structural changes occur in many of the ganglion cells of the fifth abdominal ganglia of Cambarus three to seven days after the connectives are severed between the fourth and fifth, and the fifth and sixth ganglia.

2. A smaller number of ganglion cells in the fourth and sixth ganglia likewise degenerate.

3. Complete degeneration of many of the cells occurred twenty eight to thirty-nine days after the nerve fibers were severed. The length of time required for degeneration varies.

4. The histological changes accompanying degeneration in these nerve fibers and nerve cells are similar to those which have been described by others for the nerve fibers and nerve cells of vertebrates, excepting those which pertain to the medullary sheath, which is absent in Cambarus.

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THE DEVELOPMENT OF THE SYMPATHETIC NERVOUS SYSTEM IN MAMMALS

ALBERT KUNTZ

From the Laboratories of Animal Biology of the State University of Iowa

WITH EIGHTEEN FIGURES

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I. INTRODUCTION

The present investigation of the development of the sympathetic nervous system in mammals was carried on in the laboratories of Animal Biology of the State University of Iowa, under the direction of Prof. Gilbert L. Houser.

Although much excellent work has been done on the development of the sympathetic nervous system, our knowledge concerning the sympathetic neurones and the relation of the sympathetic to the central nervous system is still very meager. Our newer conceptions of nerve-components and of the functional divisions of the peripheral nervous system call for a re-investigation of the development of the sympathetic system in order to bring this division of the nervous system into harmony with established facts.

The present investigation was undertaken in order to further exact knowledge concerning the histogenesis of the sympathetic system, to establish the histogenetic relationships between the sympathetic neurones and the neurones in the central nervous system, and to correlate the sympathetic system with the other functional divisions of the nervous system. The most important results achieved pertain to increased knowledge concerning the histogenesis of the sympathetic system and its relation to the central nervous system, and to the fact that the cardiac plexus and the sympathetic plexuses in the walls of the visceral organs are not derived from the sympathetic trunks, as has hitherto been supposed, but have their origin in nervous elements which migrate from the vagus ganglia and the walls of the hind-brain along the fibers of the vagi. During the progress of the work, two preliminary papers were published (see Bibliography).

It is a real pleasure to express my deep sense of obligation to Prof. Houser for his many helpful suggestions and for the inspiration afforded by the constant enthusiastic interest manifested by him during the progress of this investigation. I desire also to express my indebtedness to Dr. F. A. Stromsten for many valuable suggestions in technique.

II. HISTORICAL SURVEY

The earliest observations on development of the sympathetic nervous system are those of Remak ('47). That pioneer among the investigators of the sympathetic system described the anlagen of the sympathetic trunks in the chick as ganglionic enlargements on the communicating rami, situated at their point of deviation from the spinal nerves. He believed that the cells composing these ganglionic enlargements are derived from preformed elements arising in the mesoderm. This view of the mesodermal origin of the sympathetic nervous system held undisputed sway for more than two decades, and has found advocates, among whom may be mentioned Paterson ('91), in more recent times.

The work of Balfour ('77) marks the beginning of our modern conception of the ectodermal origin of the sympathetic nervous system. According to his observations on the selachians, the anlagen of the sympathetic trunks arise as simple enlargements on the spinal nerve-trunks. Subsequently, these enlargements advance toward the aorta, each, however, retaining connection with its respective nerve by a fibrous branch which becomes the communicating ramus. These ganglionic enlargements are at first independent of each other, but become united later by longitudinal commissures. These observations on the selachians were substantiated by Onodi ('86), Van Wijhe ('89), and Hoffmann ('99).

Schenck and Birdsall ('78) extended the conception of Balfour, somewhat modified, to the higher vertebrates. Tracing the development of the sympathetic trunks in birds and mammals, they found that before the anlagen of the sympathetic trunks appear, the spinal ganglia are not sharply limited distally. Groups of cells become detached from the distal ends of the spinal ganglia and advance far into the spinal nerve-trunks. These cells, they believe, constitute the anlagen of the sympathetic trunks, but they have given no clear conception of the process by which these cells are transferred from the spinal ganglia to their new location in the sympathetic anlagen.

Kölliker ('97) adopted the doctrine of Balfour and attempted to extend it to the peripheral sympathetic plexuses. In the ab-

sense of confirmatory evidence, he set up the hypothesis that the peripheral sympathetic plexuses arise as cellular offshoots from the cerebro-spinal ganglia.

Ónodi ('86) finally established the cerebro-spinal origin of the sympathetic trunks and the prevertebral plexuses for all vertebrates. He believed that the cells at the distal ends of the spinal ganglia are forced to advance farther peripherally by the pressure exerted by the newly formed elements back of them. He could not, however, derive the sympathetic trunks and the peripheral sympathetic plexuses from the same source because he found no cellular connections between these two complexes. He believed it necessary, therefore, to cling to the doctrine of Remak with regard to the peripheral sympathetic plexuses, and derive them from the mesoderm.

His ('90) introduced a new factor in the development of the sympathetic nervous system. In a human embryo 6.9 mm. in length, he observed cells migrating from the spinal ganglia. These he described as germinal cells which break through the motor roots of the spinal nerves and migrate in swarms toward the future location of the sympathetic trunks.

Pushing on the way indicated by his father, His, Jr., ('91) traced the origin of the entire sympathetic system to the spinal ganglia. He described cell-swarms in the chick similar to those described by His, Sr., in the human embryo. These cell-swarms become detached from the spinal ganglia, break through the motor roots of the spinal nerves, and migrate toward the dorso-lateral surfaces of the aorta, where they become aggregated into cell-groups which constitute the anlagen of the sympathetic trunks.

From these aggregates, cells proceed round the aorta until the latter is surrounded ventrally by a complete ring of sympathetic cells. This ring gives rise to new cell-swarms which migrate farther peripherally and become the anlagen of the peripheral sympathetic plexuses, including the sympathetic plexuses in the walls of the digestive tube and the sympathetic components related to the vagi.

In his later researches on embryos of the chick, His, Jr., ('97) found that the earliest anlagen of the sympathetic system arise about the beginning of the fourth day of incubation as a pair of longitudinal cell-columns lying along the sides of the dorsal sur-

face of the aorta. These are the beginnings of the primary sympathetic trunks. About the beginning of the sixth day the anlagen of the secondary, or permanent, sympathetic trunks arise as a series of cell-aggregates situated just median to the ventral roots of the spinal nerves. The cells giving rise to the primary sympathetic trunks migrate thither from the spinal ganglia, along the spinal nerves and the communicating rami. The anlagen of the secondary sympathetic trunks are separated from the spinal ganglia only by the fibers of the ventral nerve-roots. Neuroblasts may be found in the ventral nerve-roots, caught apparently in migration from the spinal to the sympathetic ganglia. After the appearance of the secondary trunks, the primary sympathetic trunks become resolved into the various ganglia and nerves of the prevertebral and the peripheral sympathetic plexuses. This view was adopted by Lillie ('08).

Marshall ('93) found that the anlagen of the sympathetic trunks in embryos of the frog and the chick arise "as a series of outgrowths from certain of the cranial and all of the spinal nerves." These outgrowths develop into ganglionic enlargements which become connected later by longitudinal commissures. These findings agree essentially with Balfour's observations on the selachians, but differ very materially from the findings of later observers for amphibians and birds.

In his later researches on the urodeles, Hoffmann ('02) found conditions of development differing widely from those in selachians. In this type the anlagen of the sympathetic trunks arise as scattered cells along the sides of the dorsal surface of the aorta, some of which are connected with the communicating rami by slender protoplasmic processes.

In his work on the common toad, Jones ('05) pointed out a notable difference in the development of the anterior and the posterior regions of the sympathetic trunks. In the region anterior to the second spinal nerve, they arise from cells scattered in the mesoderm. This agrees essentially with the findings of Hoffmann. In the region posterior to the second spinal nerve, ridges of cells appear along the sides of the aorta. The cells at the tops of these ridges become differentiated to form the sympathetic trunks. These findings have not been substantiated by other observers.

Kohn ('05, '07) describes the anlagen of the sympathetic trunks in the rabbit as a pair of columns of cell-aggregates arising along the sides of the dorsal surface of the aorta. Similar cells are found in intermediate positions between these cell-aggregates and the spinal nerves, in the paths later occupied by the fibers of the communicating rami. According to Kohn, the sympathetic anlagen are composed of cells which arise by the division of elements which have not migrated thither, but were differentiated in situ in the spinal nerves. Embryonal neurocytes deviate from the course of the spinal nerves toward the aorta. By division they yield a syncytial cellular communicating ramus which extends toward the aorta. Cell-groups become separated from its distal end and give rise to the cell-aggregates of the sympathetic anlagen.

According to Neumayer's observations on embryos of Lacerta (spec?) and the chick ('06), the anlagen of the sympathetic trunks arise as short cellular outgrowths on the spinal nerves which early develop ganglionic enlargements at their distal ends, which become united later by longitudinal cellular commissures. Neumayer, like Kohn, traces the origin of the sympathetic system directly to the spinal nerves. He is of the opinion that in all vertebrates the sympathetic anlagen arise from cells which are to be regarded as the offspring of the dorsal and the ventral nerve-roots and are differentiated in situ, like the cells of the spinal ganglia and the fibers of the nerve-roots.

The work of Froriep ('07) on embryos of Torpedo and of the rabbit, marks a decided advance in our knowledge of the histogenesis of the sympathetic nervous system. He succeeded in tracing medullary cells peripherally along the ventral roots of the spinal nerves. These cells he formerly interpreted as elements which give rise to the neurilemma. After Harrison ('04) showed experimentally that in amphibians the cells giving rise to the neurilemma of both the sensory and the motor fibers have their origin in the neural crest, Froriep concluded that the cells migrating peripherally in the ventral nerve-roots, either alone or with cells which wander out from the spinal ganglia, give rise to the sympathetic nervous system. In his summary he expresses the opin-

ion that all the sympathetic neurones in the sympathetic trunks as well as in the prevertebral and the peripheral sympathetic plexuses have their origin in the ventral half of the neural tube.

Held ('09) and Marcus ('09) have recently taken exception to Froriep's conclusions. Held has attempted to show, for the entire vertebrate series, that the cells present in the motor nerve-roots play no part in the development of the sympathetic system. He still regards the sympathetic system as an offshoot from the spinal ganglia. Marcus has attempted to show that the cell-groups which Froriep observed in the ventral roots of the spinal nerves do not wander out from the neural tube, but migrate thither from the neural crest. In early stages of embryos of Torpedo, he has observed cell-chains connecting the neural crest with the cell-aggregates in the ventral nerve-roots. He concludes, therefore, that the neural crest represents the sole source of all the cells giving rise to sympathetic neurones.

This brief review of the literature has shown that the advocates of the theory of the ectodermal origin of the sympathetic nervous system agree in tracing the origin of the cells giving rise to the sympathetic anlagen to the cerebro-spinal system. There is a wide difference of opinion, however, concerning the immediate source and the histogenesis of these cells.

Two views have been prevalent among the older investigators. Ónodi advanced the idea that the cells at the distal ends of the spinal ganglia are forced to advance farther peripherally by the pressure exerted by the newly formed elements back of them. In this manner cell-groups become constricted off from the spinal ganglia and give rise to the anlagen of the sympathetic trunks. His, His, Jr., and some of the later writers have traced the origin of the cells giving rise to the sympathetic anlagen to the spinal ganglia, but have accounted for the transfer of these elements from the spinal ganglia to their new location by active migration, either directly through the mesenchyme or along the paths of the spinal nerves and the communicating rami.

The difference between these two views may be accounted for in part by fundamental differences in the morphogenesis of the sympathetic nervous system in the various classes of vertebrates.

In the selachians the anlagen of the sympathetic trunks arise as ganglionic enlargements on the spinal nerves (Balfour, Van Wijhe, Hoffmann). In the amphibians fibers are present in the communicating rami before the anlagen of the sympathetic trunks appear. The latter arise along the sides of the dorsal surface of the aorta (Hoffmann, Neumayer). In Lacerta (spec?), a reptilian type, the sympathetic anlagen arise as short cellular outgrowths on the spinal nerves, which early show ganglionic enlargements at their distal ends (Neumayer). In birds the primary sympathetic trunks arise as a pair of cell-columns lying along the sides of the dorsal surface of the aorta. These early give way to the secondary sympathetic trunks which arise as cell-aggregates just median to the ventral roots of the spinal nerves (His, Jr., Lillie). In mammals the sympathetic trunks arise as a pair of cell-columns lying along the sides of the dorsal surface of the aorta (Paterson, His, Jr., Kohn).

With these morphogenetic differences in mind, it is apparent that the view of Onodi was based primarily on the selachians, while the theory of the active migration of sympathetic cells finds its basis primarily in birds and mammals.

Kohn and Neumayer have rejected both these views. They admit of no active cell migration, but trace the origin of the sympathetic system to elements which arise in situ in the spinal nerves, and account for the multiplication of cells along the paths of the communicating rami and in the sympathetic anlagen by local cell division. Their views, however, are obviously influenced by their conception of the neurone and their allegiance to the theory of local differentiation and the multicellular nature of nerve-fibers.

III. METHODS OF INVESTIGATION

The following observations are based on embryos of the pig. Several embryos of the cat and a goodly number of embryos of the chick were at my disposal and were used for checking results. Embryos of the pig were found to be more desirable than embryos of the cat, because the cells in the former are comparatively larger and appear to be less crowded.

Various methods of technique were employed, but the iron-hæmatoxylin method was found to be most satisfactory. The embryos were fixed in Zenker's fluid, chrom-aceto-formaldehyde, or chrom-oxalic acid. The sections were usually cut a thickness of 10 microns. It was found most satisfactory to over-stain in hæmatoxylin, then to differentiate in the iron-alum bath until the color had almost disappeared except from the denser tissues, and to counter-stain lightly with orange-G. The degree to which the hæmatoxylin shall be differentiated in order to obtain the best results must be learned by experience.

The results obtained from one lot of embryos may be of interest to students of special technique. A few young embryos taken from the laboratory collection were sectioned and stained. These had been kept in 10 per cent formaldehyde for some ten years. When stained by the iron-hæmatoxylin method, the nuclei of the nerve cells took a much deeper and more solid stain than in fresh material. These preparations were, therefore, found very serviceable in tracing migrant nervous elements.

The silver reduction method was used with good results in the later stages.

IV. OBSERVATIONS

I. SYMPATHETIC TRUNKS

(*a*) *Early development.*—The earliest traces of the sympathetic trunks appear in the thoracic region of embryos of the pig about 6 mm. in length, as small cell-aggregates lying along the sides of the dorsal surface of the aorta. The spinal nerves in the thoracic region have already become fibrous and extend peripherally to a point a little beyond the dorsal level of the aorta. Fibrous communicating rami are not present as yet, and the sympathetic anlagen are apparently independent of the spinal nerves.

In embryos 7 mm. in length, the anlagen of the sympathetic trunks may be traced throughout the thoracic and the dorsal region. The cell-aggregates have become larger, and because of the strong curvature of the embryo they are brought into such

close proximity with each other that the entire anlage of the sympathetic trunk appears as a continuous cell-column. This cell-column is not of uniform diameter, but in transverse sections traces of it are not wanting in any section in the thoracic and the dorsal region. Fibers are not present as yet either in the anlagen of the sympathetic trunks or in the communicating rami. The sympathetic anlagen are essentially cellular. The cells are closely aggregated and many of them present delicate protoplasmic processes, or are included in small syncytia. These structures are not very apparent in transverse sections, but in sagittal or frontal sections the anlagen of the sympathetic trunks present the appearance of a loose-meshed cellular network.

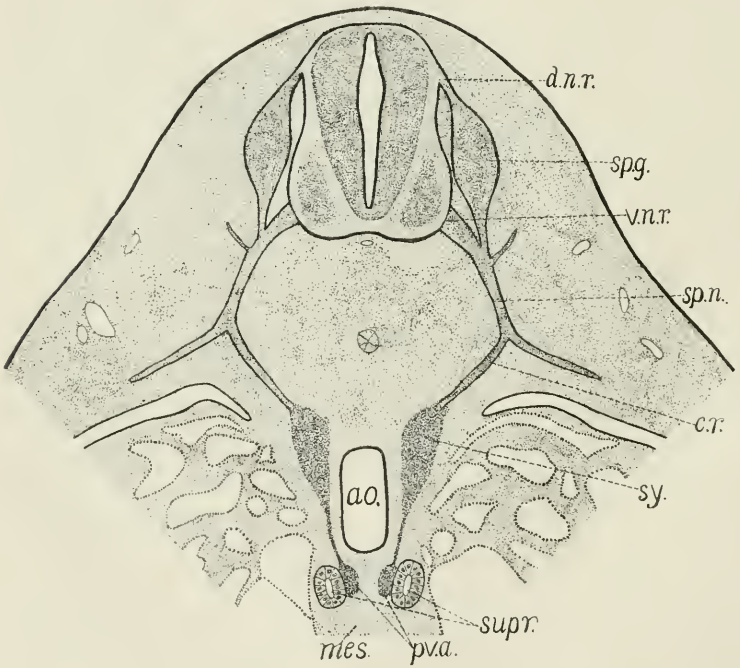

Fig. *A*. Diagrammatic transverse section of an embryo 12 mm. in length through the suprarenal bodies, × 50.

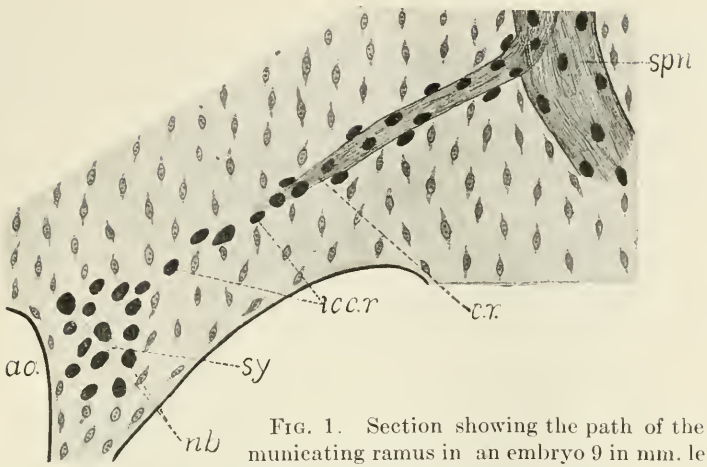

FIG. 1. Section showing the path of the communicating ramus in an embryo 9 in mm. length, × 210. (See reference letters below.)

REFERENCE LETTERS OF FIGURES

(*Except Fig. 16*)

All of the figures were drawn with the aid of the camera lucida or the projection lantern. A uniform scale of magnification was not adopted, but the scale of diameters of the drawing as reproduced is given in the description of each figure.

a.i.c.–Accompanying indifferent cells.
ao.–Aorta.
b.rec.–Branch of recurrent nerve.
ca.–Carotid artery.
car.n.–Cardiac nerves.
car.b.–Anlagen of cardiac plexus.
c.r.–Communicating ramus.
c.m.d.n.r.–Cells migrating into dorsal nerve-root.
c.m.pv.–Cells migrating from sympathetic trunks into prevertebral plexuses.
c.m.v.r.–Cells migrating into ventral nerve-root.
c.m.vag.r.–Cells migrating into vagus rootlets.
d.n.r.–Dorsal nerve-root.
e.l.m.–External limiting membrane.
f.pv.–Fibers extending into prevertebral plexuses.
g.c.–Germinal cells of His.
i.c.c.r.–Indifferent cells in communicating ramus.
i.l.m.–Internal limiting membrane.
mes.–Mesentery.
m.r.f.–Motor root-fibers.
m.s.p.–Anlage of myenteric and submucous plexuses.
nb.–Neuroblasts.
oe.–Œsophagus.
o.rec.n.–Origin of recurrent nerve.
p.a.–Pulmonary artery.
pv.a.–Anlagen of prevertebral plexuses.
rec.n.–Recurrent nerve.
sp.g.–Spinal ganglion.
sp.n.–Spinal nerve.
s.r.f.–Sensory root-fibers.
supr.–Suprarenal bodies.
sy.–Anlagen of sympathetic trunks.
t.–Trachea.
vag.–Vagus trunk.
vag.b.–Branch of vagus nerve.
vag.r.–Rootlets of vagus nerve.
v.n.r.–Ventral nerve-root.

The spinal nerves are composed of bundles of parallel fibers accompanied by numerous cells which, as will be shown presently, are obviously of medullary and ganglionic origin. These cells are present both at the surface of the bundles and among the growing fibers in the interior of the nerve-trunk. They are easily distinguished from the cells of the surrounding mesenchyme by their larger size and the characteristic chromatin structure of their nuclei.

In embryos 9 mm. in length, the anlagen of the sympathetic trunks may be traced from the cervical to the sacral region. The

Fig. 2. Section showing the path of the communicating ramus in an embryo 10 mm. in length, × 200.

cells have become more numerous and more closely aggregated. Fibers are present in the communicating rami, but do not yet extend into the anlagen of the sympathetic trunks (fig. 1, c.r.). "Accompanying" cells are present all along the spinal nerves and the communicating rami. At the tips of the growing rami, cells appear to become detached and to wander into the anlagen of the sympathetic trunks in advance of the growing fibers (fig. 1, i.c.c.r.).

In embryos 10 mm. in length, the fibers of the communicating

rami extend into the anlagen of the sympathetic trunks (fig. 2, c.r.). It may be noted in passing that in some embryos 10 mm. in length the number of cells accompanying the fibers of the spinal nerves has materially decreased, while in other embryos of the same length, the "accompanying" cells are equally as numerous as in the preceding stages. We may conclude, therefore, that

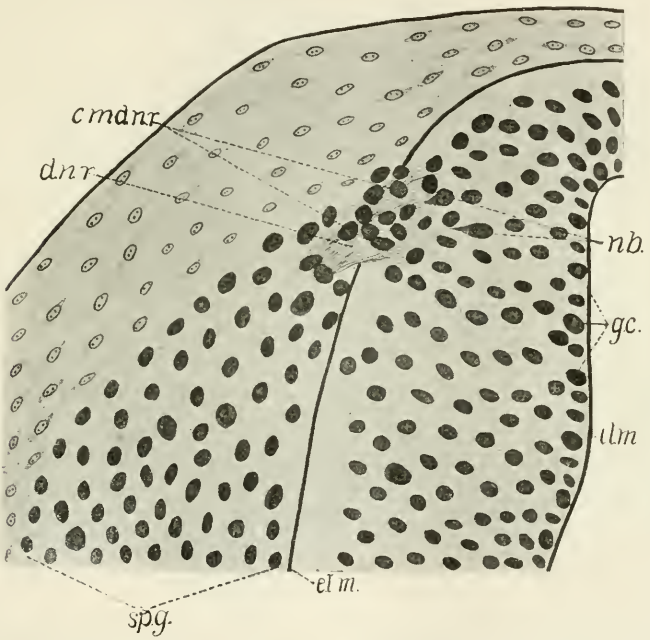

FIG 3. Transverse section of the dorsal part of the neural tube of an embryo 7 mm. in length, showing cells migrating into the dorsal nerve-root, × 275.

there is a notable degree of individual variation in the rate of development.

(*b*) *Cell migration.*—In a recent paper[1] the writer has described the migration of nervous elements from the neural tube and the spinal ganglia, along the spinal nerves and the communicating rami, into the anlagen of the sympathetic trunks. The earliest

[1] A contribution to the histogenesis of the sympathetic nervous system. *Anatomical Record*, vol. 3, no. 8, pp. 458–465.

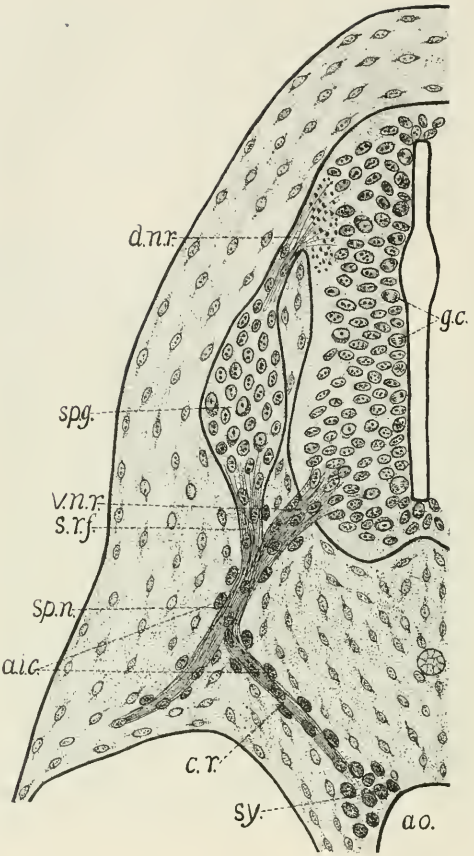

FIG. 4. Transverse section of the neural tube and the anlage of the sympathetic trunk of an embryo 11 mm. in length, × 125.

evidence of the migration of medullary cells from the neural tube is found in embryos about 4.5 mm. in length. At this stage the neural crest is not yet differentiated into ganglia, but appears as an inconspicuous ridge spreading laterally from the median dorsal line of the neural tube. It is so inconspicuous indeed that in many sections it may be distinguished only under favorable conditions. In a few instances the fibers of the ventral nerve-roots have penetrated the walls of the neural tube and are accompanied

by medullary cells which have broken through the external limiting membrane.

In embryos 7 mm. in length, the spinal ganglia are distinct, but are not completely formed as yet, and have receded but a short distance from the point at which the fibers of the dorsal nerve-roots enter the neural tube. In transverse sections, numerous breaches may be observed in the external limiting membrane in

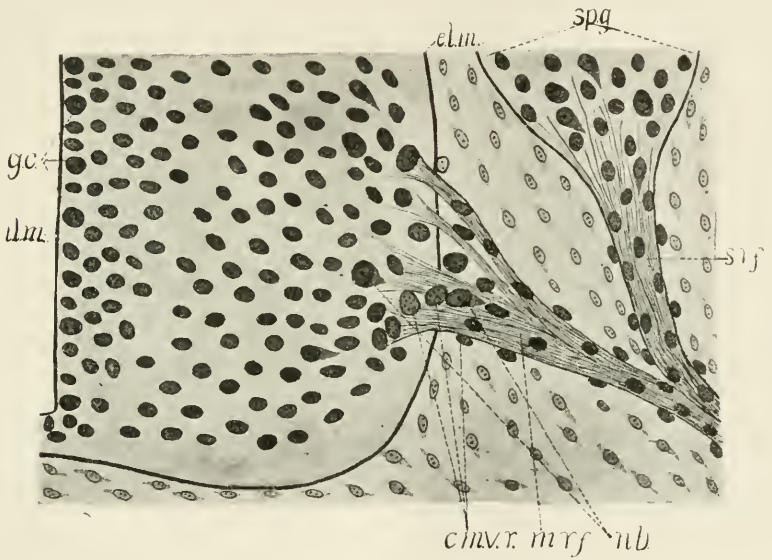

FIG. 5. Transverse section of the ventral part of the neural tube of an embryo 9 mm. in length, showing cells migrating into the ventral nerve-root. × 250.

the region of the dorsal nerve-roots. Rows of cells practically touching each other end to end may be traced from the mantle layer, through these breaches, into the proximal parts of the dorsal nerve-roots (fig. 3, c.m.d.n.r.). Further evidence for the migration of medullary cells into the dorsal nerve-roots is presented by the fact that in many sections where no breaches occur, cells are crowded close to the external limiting membrane in this region. In embryos 9 mm. and over in length, this area is always

occupied by the fibers of the dorsal nerve-roots, and cells are rarely found among them (fig. 4, d.n.r.). Migration of medullary cells into the dorsal nerve-roots evidently ceases before the 9 mm. stage is reached. It is probably only a transient process which takes part in the development of the cerebro-spinal ganglia.

In transverse sections of embryos from 6 to 11 mm. in length, breaches occur frequently in the external limiting membrane in the region of the ventral nerve-roots. Medullary cells migrate in considerable numbers, through these breaches, into the ventral nerve-roots (fig. 5, c.m.v.r.). This fact has recently been observed by Carpenter and Main ('07). I have been able to substantiate

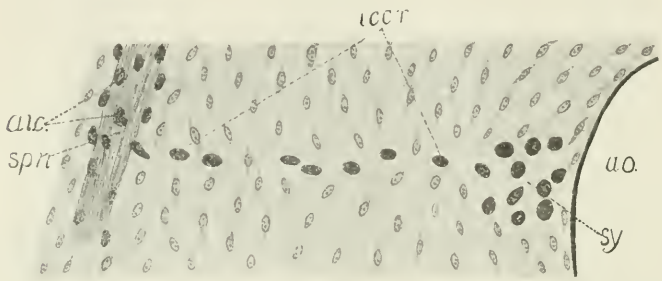

Fig. 6. Section showing the path of the communicating ramus in an embryo 7 mm. in length, × 190.

their observation that cells may be observed "just inside the external limiting membrane, in an intermediate position half in and half out of the neural tube, and in the base of the ventral nerve-root just outside the external limiting membrane."

The orientation of the cells in the neural tube is such, during the period of migration, that the cells which migrate into the dorsal and the ventral nerve-roots seem to have their origin in more or less distinct regions. In the dorsal region most of the migrating cells move quite directly outward from the dorsal zone, while others tend dorso-laterally in slight curves from regions ventral to the dorsal nerve-roots. In the ventral region some of the

migrant cells move quite directly outward from the ventral zone, while others tend ventro-laterally from the region in which later the lateral horn of the gray matter arises.

These migrant medullary cells, with similar elements which wander out from the spinal ganglia, migrate peripherally along the fibers of the spinal nerves. In transverse sections of embryos 7 or 8 mm. in length, the spinal ganglia are not sharply limited distally. Cells become separated from their distal ends and migrate peripherally along the fibers of the sensory roots. There is no recognizable difference between the cells which wander down from the spinal ganglia and those which migrate out from the neural tube. It is impossible, therefore, to distinguish the cells which migrate from the neural tube along the ventral nerve-roots from those which become separated from the spinal ganglia, after they have advanced beyond the point of union of the sensory and motor roots. These "accompanying" cells are present in the spinal nerve-trunks as far as the latter may be traced. Fibers are not present as yet in the communicating rami, but at a point a little above the level of the aorta, cells, either singly or in small groups, deviate from the course of the spinal nerves nearly at right angles and migrate through the mesenchyme toward the dorso-lateral surfaces of the aorta, along the paths later occupied by the fibers of the communicating rami (fig. 6, i.c.c.r.). These findings do not differ essentially from those of Froriep. They differ from those of the older investigators primarily in the fact that cells migrate peripherally not only from the spinal ganglia but also from the neural tube along the fibers of the ventral nerve-roots.

It is important to note at this point that the cells accompanying the fibers of the spinal nerves actually migrate peripherally. Fig. 7 indicates schematically the course and the direction of the cells which migrate along the spinal nerves and the communicating rami into the sympathetic anlagen. Fig. 8 is designed to show approximately the relative number of "accompanying" cells present in the spinal nerves in successive stages during the period of migration, and also the relative number remaining in the nerve-trunks after migration has ceased. The figures in the hori-

zontal line represent the lengths of the embryos in mm.; the figures in the vertical line indicate the number of cells present in a given length of longitudinal sections of the spinal nerves, as they appear in transverse sections of the embryos, taken at random between the point of union of the sensory and the motor roots and the origin of the communicating rami. Embryos which seemed to be most normal in their development were selected, and the curve is based on the averages of ten independent counts. This curve

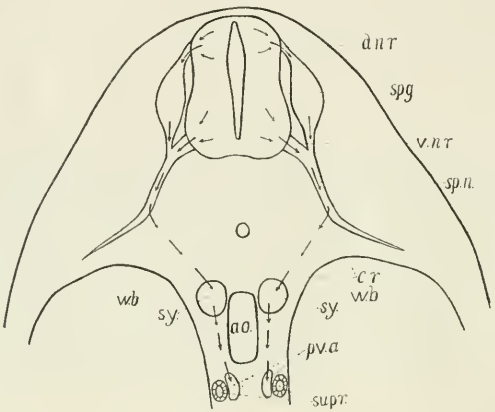

FIG. 7. Diagrammatic transverse section of an embryo 9 or 10 mm. in length. The arrows indicate the course and the direction of the cells migrating from the neural tube and the spinal ganglia into the sympathetic anlagen.
ao., Aorta. *c.r.*, Path of communicating ramus. *d.n.r.*, Dorsal nerve-root. *pv.a.*, Anlagen of prevertebral plexuses. *sp.g.*, Spinal ganglion. *sp.n.*, Spinal nerve. *supr.*, Suprarenal bodies. *sy.*, Sympathetic trunks. *v.n.r.*, Ventral nerve-root. *w.b.*, Wolffian body.

indicates that the rate of migration reaches its maximum in embryos 9 mm. in length, and that migration practically ceases when a length of 13 mm. is attained. It also indicates that a relatively small but fairly constant number of cells remains in the spinal nerves after migration has ceased.

As already indicated in reviewing the literature, Kohn and Neumayer have attempted to account for the cells giving rise to the sympathetic nervous system, by local differentiation of elements

already present in the sensory and the motor nerve-roots. This view seems to be quite generally accepted by the advocates of the theory of local differentiation and the multicellular nature of nerve-fibers. These two conceptions obviously go hand in hand. It is not the writer's purpose to discuss the nature of nerve-fibers. Suffice it to say that in the light of the recent investigations of Cajal, Harrison, and others, the neurone theory

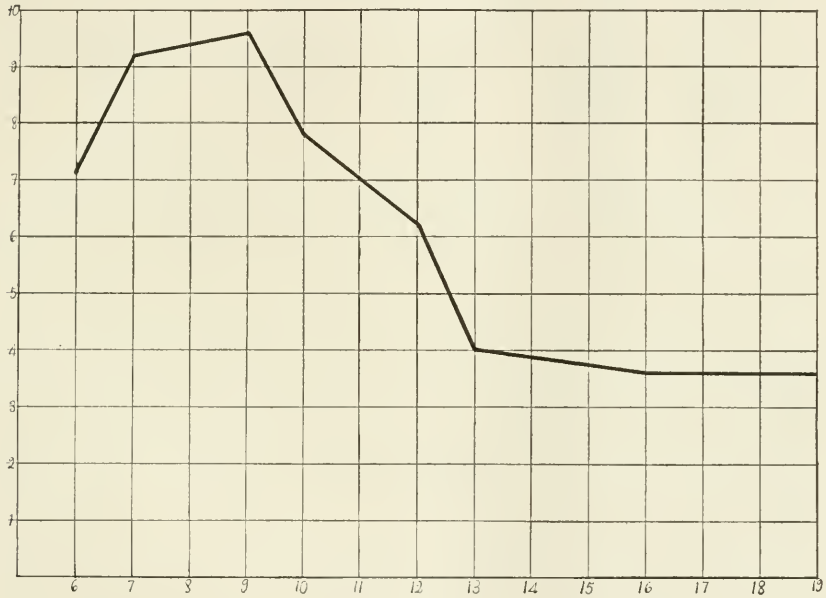

FIG 8. Curve designed to indicate the relative rate of migration of cells from the neural tube and the spinal ganglia along the spinal nerves, in successive stages of development. For explanation see text.

seems to be firmly established. On the other hand, if the "accompanying" cells do not migrate peripherally we cannot account for the rapid decrease in the number of such cells present in the proximal part of the spinal nerves, which, as shown in fig. 8, takes place in embryos from 9 to 13 mm. in length. Mitotic figures occur occasionally in the nerve-roots as well as in the nerve-trunks and in the communicating rami. Doubtless, many

of the "accompanying" cells arise by the mitotic division of "indifferent" cells along the course of migration, but these mitotic figures are by no means sufficiently numerous to account for the multitudes of cells which take part in the development of the sympathetic trunks. Furthermore, I have observed cells along the spinal nerves and the communicating rami, which, as will be shown later, are neuroblasts. Such cells were recently described by Cajal ('08) in the spinal nerves, and the communicating rami in embryos of the chick. According to Cajal, "these neuroblasts do not correspond to the neurocytes of Kohn, but to the real motor cells in the neural tube." These facts are incompatible with the theory of local differentiation.

(*c*) *Later development.*—In embryos 12 mm. in length, the anlagen of the sympathetic trunks are rapidly becoming fibrous. They still appear as continuous cell-columns showing little evidence of their future segmental character. The earliest fibers of the longitudinal commissures, therefore, do not grow out through the inter-gangliar spaces, but the cells become aggregated into distinct ganglia after the sympathetic trunks have become fibrous. "Accompanying" cells are still present along the spinal nerves and the communicating rami, but they are notably fewer than in the preceding stages. Cells may still be observed migrating from the neural tube and the spinal ganglia, but such migration probably does not continue far beyond this stage. In embryos over 12 mm. in length, the motor niduli are sharply limited, and medullary cells are rarely seen along the fibers of the ventral nerve-roots as they traverse the marginal veil. The spinal ganglia are also becoming more sharply limited distally, and cells no longer become separated from them. The later development of the sympathetic trunks consists in progressive changes and growth of the elements already present.

(*d*) *Nature of migrating cells.*—In his excellent work on the earliest differentiations in the central nervous system, Schaper ('97) made a most thorough and detailed study of the cells which arise by the mitotic division of the "germinal" cells (Keimzellen) of His. These cells were originally described by His as cells

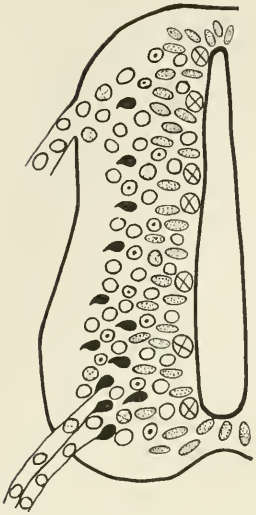

Fig. 9

Fig. 10

Fig. 9. Diagram illustrating the developmental relationships of the neuroblasts and the embryonic supporting cells in the neural tube of mammalian embryos (modified from Schaper, '97). Elongated dotted cells = ependymal cells; large circular cells with crosses = germinal cells of His; plain rounded cells = indifferent cells; rounded cells with dotted crosses = indifferent cells which undergo further division by mitosis; rounded cells with dots in center = neuroglia cells; black cells = nerve cells.

Fig. 10. Ganglion cells, neuroblasts, and indifferent cells, × 1100. *a.*, Cells in the spinal ganglia. *b.*, Neuroblasts in the ventral nerve-roots. *c.*, Neuroblasts in the spinal nerve trunks. *d.*, Bundles of fibers with accompanying indifferent cells, from the spinal nerves. *e.*, Neuroblasts in the communicating ramus. *f.*, Neuroblasts in the anlagen of the sympathetic trunks.

of ectodermal origin undergoing mitotic division near the internal limiting membrane of the embryonic neural tube, giving rise to cells which develop into neuroblasts. Schaper has shown that the cells arising by the mitotic division of the "germinal" cells of His do not all develop into neuroblasts. They are cells of an indifferent character. In the lower vertebrates they are transformed either into neuroblasts or into embryonic supporting cells. In the higher vertebrates, however, many of these "indifferent" cells retain a capacity for further propagation by di-

vision and give rise to new generations of "indifferent" cells which may develop either into neuroblasts or into embryonic supporting cells. The accompanying figure (fig. 9), modified from Schaper, has been introduced to illustrate the developmental relationships of the neurones and the supporting elements in the embryonic nervous system. According to Schaper's original description, the "indifferent" cells are characterized by large rounded nuclei showing a delicate chromatin structure, and very little cytoplasm. The "neuroblasts" are characterized by large rounded nuclei showing little structure in the interior except a well defined nucleolus, and a larger cytoplasmic body which is early drawn out to a point at one side.

The great majority of the cells migrating from the neural tube and the spinal ganglia, along the spinal nerves and the communicating rami in embryos of the pig, answer to the description of the "indifferent" cells of Schaper. When observed in the motor niduli or in the distal ends of the spinal ganglia, their nuclei usually appear more or less rounded in outline and show a very delicate chromatin structure. The cytoplasm is so meager that it can be observed only under the most favorable conditions. As these cells migrate peripherally they assume a more elongated form. In the ventral nerve-roots many of them have assumed their maximum elongation soon after they have left the neural tube. In the lines of cells which may be observed migrating out of the neural tube, the inner ones are often nearly circular in outline, the outer ones are greatly elongated, while those in intermediate positions show varying degrees of elongation. This elongation cannot be accounted for mechanically, as by the squeezing through a narrow aperture in the external limiting membrane. These apertures are usually broad enough to permit free passage of the cells. It is probably due to more subtle forces which are operative in the process of migration. Furthermore, it is by no means certain that such a change of shape always takes place. Rounded "indifferent" cells are sometimes observed far distally along the course of migration, while elongated cells are present in the motor niduli and in the distal ends of the spinal ganglia.

Among the "indifferent" cells of Schaper, cells are occasionally found which are characterized by large rounded or elongated nuclei showing a well defined nucleolus and very little chromatin structure, and a considerable quantity of cytoplasm which is usually drawn out to a point at one side (fig. 10). These cells are obviously the "neuroblasts" of Schaper. They are few in number, but occur all along the path of migration of the sympathetic cells. I have observed them in the ventral nerve-roots both inside and outside the external limiting membrane, in the spinal nerves, in the communicating rami, and in the anlagen of the sympathetic trunks.

The histogenetic relationships of the cells taking part in the development of the sympathetic trunks will be considered further in section V. The facts of importance at this point are that cells which are endowed with a capacity to develop into neurones, migrate peripherally from the neural tube and the spinal ganglia, and that some of these cells migrate into the anlagen of the sympathetic trunks. These facts establish a direct genetic relationship between the sympathetic and the central nervous system. We are not to suppose, however, that all the cells taking part in the development of the sympathetic trunks actually migrate as such from their sources in the neural tube and the spinal ganglia. Doubtless, many arise by the mitotic division of "indifferent" cells along the course of migration. The sources of these migrating elements are, therefore, sufficient to account for all the cells which take part in the development of the sympathetic trunks and the sympathetic plexuses genetically related to them.

II. PREVERTEBRAL PLEXUSES

(*a*) *Development.*—In embryos 10 mm. in length, the anlagen of the prevertebral plexuses may be recognized as small cell-aggregates lying along the ventro-lateral surfaces of the aorta in the dorsal and the lumbar regions. In these regions the sympathetic trunks are not sharply limited ventrally. Cells become separated from their ventral margins and migrate ventrally along the sides of the aorta (fig. 11, c.m.pv.). In the region of the

suprarenals, such cells have descended as far as the mesial surfaces of these bodies which, at this time, appear as compact cell-columns more or less circular in transverse section, lying parallel with the aorta a short distance from its ventro-lateral surfaces (fig. 11, supr.). Posterior to the suprarenals, small cell-aggregates are present all along the ventro-lateral surfaces of the aorta as far as the origin of the iliac arteries. It is impossible, in this stage, to determine the limits of the anlagen of the several sym-

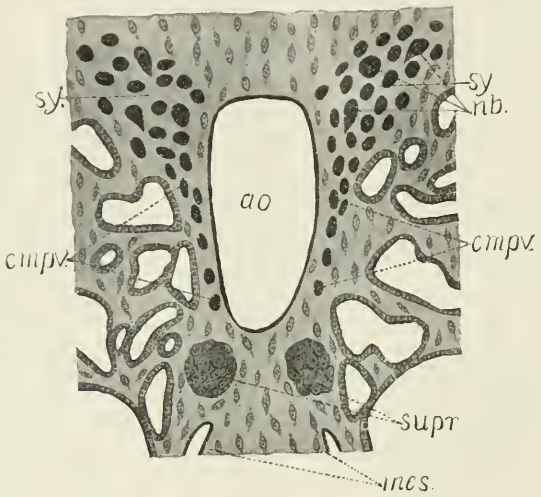

Fig. 11. Transverse section through the sympathetic trunks and the suprarenal bodies of an embryo 9 mm. in length, × 150.

pathetic plexuses in this region. The only distinction between the cell-aggregates which constitute the anlagen of the cœliac, the renal, the abdominal aortic, and the hypogastric plexuses consists in a difference in degree of development. Development proceeds somewhat more rapidly in the anterior than in the posterior region. In transverse sections through this region, traces of one or the other of these plexuses appear in nearly every section.

In embryos 13 mm. in length, the cell-aggregates lying along the ventro-lateral surfaces of the aorta have become more pronounced. The greatest development occurs in the region of the suprarenals. At a few points, fibers may be traced from the sympathetic trunks into the anlagen of the prevertebral plexuses (fig. 12, f.pv.). In the region of the cœliac plexus, fibers have advanced farther peripherally and may be traced for a short distance into the mesentery. The anlagen of the abdominal aortic plexus have developed into a loose network which completely surrounds the aorta ventrally.

In embryos 16 mm. in length, the prevertebral plexuses are becoming more distinct and more fibrous. The sympathetic trunks are more sharply limited ventrally, except in the region of the suprarenals. At this point there is still a continuous line of sympathetic cells extending from the sympathetic trunks into the cell-aggregates associated with the suprarenals.

(*b*) *Histogenetic relationships.*—The cells constituting the anlagen of the prevertebral plexuses show all the characters of the cells present in the sympathetic trunks. Continuous lines of cells may be traced from the latter into the former. The prevertebral plexuses, therefore, stand in direct genetic relationship to the sympathetic trunks. Mitotic figures occur occasionally along the courses of migration from the sympathetic trunks as well as in the anlagen of the prevertebral plexuses. Doubtless, a goodly number of the cells taking part in the development of the prevertebral plexuses arise by the mitotic division of "indifferent" cells along the courses of migration. The development of the prevertebral plexuses is, therefore, entirely analogous with the development of the sympathetic trunks.

III. VAGAL SYMPATHETIC PLEXUSES

(*a*) *Introductory.*—Under the term vagal sympathetic plexuses, we shall consider those plexuses, usually regarded as sympathetic, which are directly related to the vagi; viz., the cardiac plexus and the sympathetic plexuses in the walls of the visceral organs.

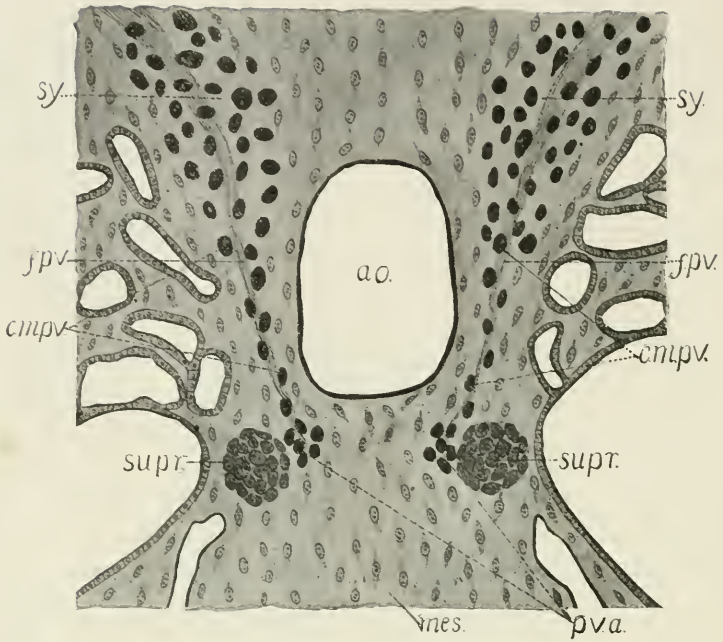

Fig. 12. Transverse section through the sympathetic trunks and the anlagen of the cœliac plexus of an embryo 12 mm. in length, × 150.

Our knowledge concerning the development of the sympathetic plexuses related to the vagi is very limited. The older workers generally gave little attention to the peripheral sympathetic plexuses. Ónodi ('86), though he traced the origin of the sympathetic trunks and the prevertebral plexuses to the spinal ganglia, could not derive the peripheral sympathetic plexuses from the same source, because he found no cellular connections between the latter and the sympathetic trunks. He believed it necessary, therefore, to cling to the doctrine of Remak ('47) with regard to the peripheral sympathetic plexuses and derive them from the mesoderm. His, Jr., ('91) traced the origin of the peripheral sympathetic plexuses, including the sympathetic plexuses in the walls of the digestive tube and the sympathetic components related to the vagi, to cell-swarms which migrate peripherally from the anlagen of the sympathetic trunks.

Later writers have generally assumed that the cardiac plexus and the sympathetic plexuses in the walls of the visceral organs have their origin in the sympathetic trunks, but the course of their development has not been made clear. The literature bearing on this point is conspicuously meager.

My own observations, as indicated in a recent paper,[2] have shown that the cardiac plexus and the sympathetic plexuses in the walls of the visceral organs do not owe their origin to the sympathetic trunks as has hitherto been supposed, but that they arise from cells which migrate from the vagus ganglia and the walls of the hind-brain along the fibers of the vagi.

Because the origin of these plexuses is distinct and separate from the origin of the sympathetic trunks and the sympathetic plexuses described above as prevertebral plexuses, they cannot properly be characterized as prevertebral sympathetic plexuses. In view of their relation to the vagi I have chosen to designate them as vagal sympathetic plexuses. The term "vagal sympathetic" is a departure from the established nomenclature, but inasmuch as there is no good collective term which could be applied to the cardiac plexus and the sympathetic plexuses in the walls of the visceral organs, it has seemed well, for the sake of clearness, to employ a new term.

(*b*) *Myenteric and submucous plexuses.*—In transverse sections of embryos 6 and 7 mm. in length, in the region of the œsophagus, the vagus trunks appear as large bundles of loosely aggregated fibers accompanied by numerous rounded or elongated cells. These cells, which, as will be shown later, are of medullary and ganglionic origin, are easily distinguished from the cells of the surrounding mesenchyme by their larger size and the characteristic chromatin structure of their nuclei. Many of them appear to become separated from the nerve-trunks and to wander into the walls of the œsophagus until the latter is completely surrounded by these migrant cells. In a few sections short fibers are seen to bend from the vagus trunks toward the œsophagus (fig. 13, vag.

[2] The rôle of the vagi in the development of the sympathetic nervous system. *Anatomischer Anzeiger*, Bd. 35, no. 15, 16, pp. 381–390.

b.). From the tips of these growing fibers, cells pass in well defined paths into the walls of the œsophagus. It is probable that most of the cells which become separated from the vagi wander out along the fibers of these growing branches. The cells which have wandered into the walls of the œsophagus are not arranged in well defined rings as yet, but are loosely scattered in the tissues (fig. 13, m.s.p.).

The fibers of the vagi do not yet extend beyond the region of the heart. In transverse sections through the stomach, the paths of the vagus branches are indicated by the presence of numerous

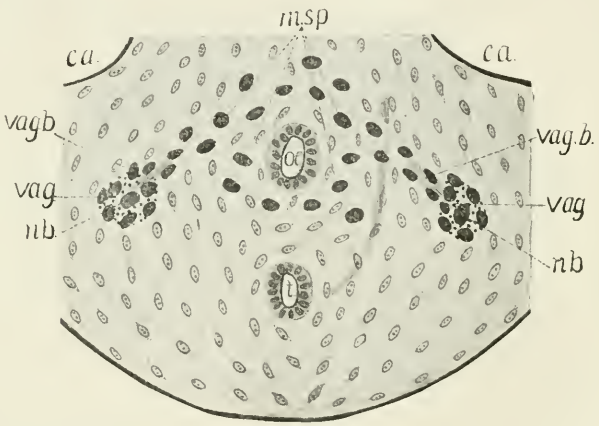

Fig. 13. Transverse section through the œsophagus and the vagus trunks in an embryo 7 mm. in length, × 160.

cells like those described above. These cells show a tendency to spread until they have completely surrounded the walls of the stomach. Similar cells are found scattered in the walls of the intestine as far as the latter can be traced. Thus, it appears that, having once become established in the anterior region of the digestive tube, these cells migrate posteriorly along its course.

That these migrant nervous elements found in the walls of the digestive tube have migrated thither from the vagus trunks cannot be doubted. There is no difficulty in tracing cells from the

tips of the growing branches of the vagi into the walls of the œsophagus. Furthermore, it is impossible to trace cells from any other source. There is no evidence as yet of the migration of cells from the sympathetic trunks or from the prevertebral plexuses toward the walls of the digestive tube. Neither cellular nor fibrous connections occur between the sympathetic trunks or the prevertebral plexuses and the sympathetic plexuses in the walls of the digestive tube until the latter have become well established.

In transverse sections of embryos 9 mm. in length, there is no evidence of cells wandering from the vagus trunks toward the œsophagus except along the fibers of the growing branches. These courses are still plainly visible. The migrant cells in the walls of the œsophagus have become arranged in more definite rings, and none are found scattered in the surrounding tissue. Numerous cells still accompany the fibers of the vagi all along their course and seem to escape freely at their growing tips.

In embryos 12 mm. in length, the number of cells in the proximal part of the vagus trunks has materially decreased. Most of those still remaining probably subserve a supporting function. The more distal parts still contain numerous cells. It is probable, however, that the migration of cells along the vagi does not continue far beyond this stage. In the region just anterior to the stomach, the vagus trunks have broken up into a loose network which is the beginning of the œsophageal plexus. Vagus fibers still accompanied by numerous cells may now be traced along the lesser curvature of the stomach. The anlagen of the cœliac plexus are well established, but there are no fibrous connections as yet between them and the anlagen of the sympathetic plexuses in the walls of the digestive tube.

In embryos 16 mm. in length, the vagus trunks as well as their branches, many of which have established connections with the myenteric and the submucous plexuses, are apparently free from migrating cells. In the walls of the œsophagus, the cells which have wandered in are aggregated into more or less distinct groups arranged in two broken rings. The myenteric and the submucous plexuses are thus becoming distinct. A similar arrangement, though less definite, is apparent also in the walls of the intestine.

Fibrous connections have become established with the sympathetic trunks as well as with the cœliac and the hypogastric plexuses. It is interesting to note that all these sympathetic nerves still contain numerous "accompanying" cells which are apparently migrating peripherally along their fibers. It is probable, therefore, that cells wander down from the sympathetic trunks into the myenteric and the submucous plexuses after these fibrous connections are established.

(*c*) *Pulmonary plexuses.*—In transverse sections of embryos 6 or 7 mm. in length, some of the cells which wander from the vagus trunks toward the œsophagus, in the region of the bifurcation of the trachea, are carried out along the anterior and the dorsal surfaces of the bronchi. These cells obviously give rise to the anlagen of the pulmonary plexuses.

(*d*) *Cardiac plexus.*—The first unmistakable evidence of ganglia pertaining to the cardiac plexus is found in embryos about 12 mm. in length. In transverse sections through the anterior region of the heart, small groups of nervous elements are observed ventral to the trachea (fig. 14, car.p.), a few of which have penetrated deep into the angle between the aorta and the pulmonary artery. These cell-aggregates constitute the anlagen of the earliest ganglia of the cardiac plexus. They are without fibrous connections as yet, but a few short fibrous branches are seen to arise from the vagus trunks and the left recurrent nerve, which extend toward the heart (fig. 14, carn). These are obviously the earliest cardiac nerves. Their fibers are still loosely aggregated and are accompanied by numerous cells, some of which appear to escape at the tips of the nerves and to migrate toward the cardiac ganglia in advance of the growing fibers. Nerves cannot be traced as yet from the sympathetic trunks toward the heart, and there is no evidence of the migration of cells from the sympathetic trunks into the anlagen of the cardiac plexus.

In embryos 16 mm. in length, branches of the vagi as well as cardiac nerves having their origin in the sympathetic trunks may be traced into the ganglia of the cardiac plexus. Here again it is interesting to note that while the branches of the vagi are apparently free from migrating cells, the cardiac nerves having their

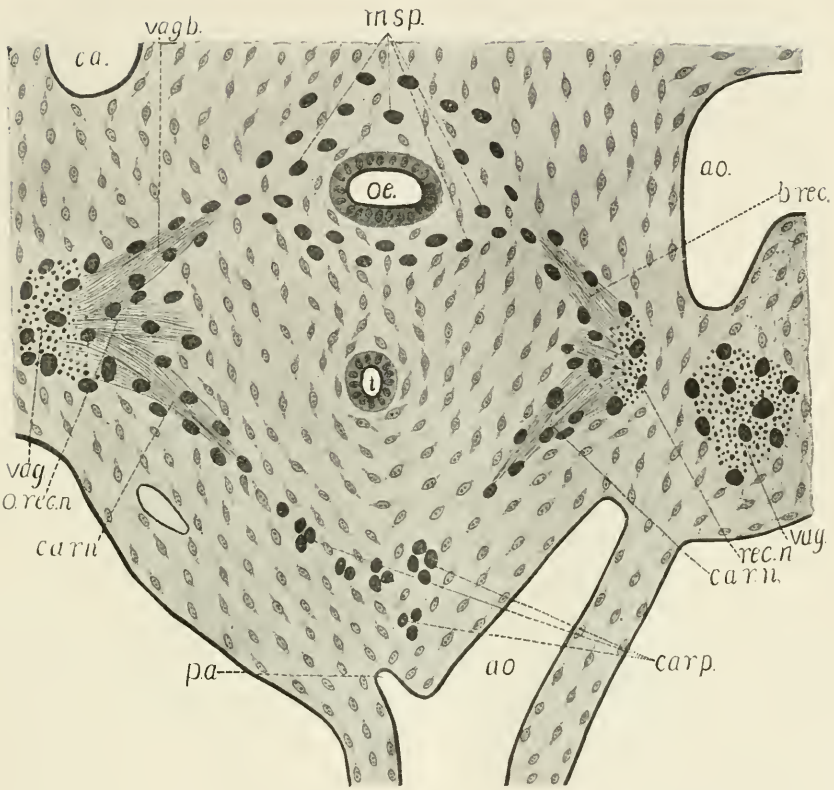

FIG. 14. Transverse section through the œsophagus, the vagus trunks, and the anlagen of the cardiac plexus in an embryo 13 mm. in length, × 100.

origin in the sympathetic trunks still contain numerous "accompanying" cells which are apparently migrating peripherally along their fibers. It is probable, therefore, that the cardiac plexus also receives cells from the sympathetic trunks after the sympathetic cardiac nerves have become established.

This stage in the pig may be compared with the human embryo 10.2 mm. in length described by His, Jr. ('91). He also observed that, in this stage, the branches of the vagi are comparatively free from cells, while the cardiac nerves having their origin in the sympathetic trunks contain many cellular elements. He con-

cluded, therefore, that the ganglia of the cardiac plexus are composed exclusively of cells which have migrated thither from the sympathetic trunks.

The above observations prove conclusively that the earliest anlagen of the cardiac plexus in the pig arise from cells which migrate thither from the vagus trunks. This is probably true for all mammals. In the human embryo of His, Jr., referred to above, the cardiac plexus already had fibrous connections with both the vagi and the sympathetic trunks. The anlagen of the cardiac plexus would probably have been found considerably earlier.

My observations on the later development of the cardiac plexus in the pig do not differ essentially from those of His, Jr.,[3] on the human embryo, except that the earliest cardiac nerves having their origin in the sympathetic trunks are less intimately associated with the vagi, and enter the cardiac plexus independently. This fact was also observed by His, Jr., in embryos of the cat.

(*e*) *Cell migration along the vagi.*—In sections taken at right angles to the axis of the trunk, in the head region of embryos 9 or 10 mm. in length, medullary cells may be observed migrating from the walls of the hind-brain into the rootlets of the vagus and the spinal accessory nerves (fig. 15, c.m.vag.r.). That these cells wander out in considerable numbers cannot be doubted. In many sections medullary cells are observed drawn out into cone-shaped heaps in the nerve-rootlets as they traverse the marginal veil. Occasionally one of these cells is observed half in and half out of the neural tube, and many are present in the nerve-rootlets just outside the external limiting membrane.

In sagittal sections the entire vagus trunk is seen to contain many of these "accompanying" cells which are apparently migrating peripherally. The ganglion of the trunk is, at this stage, a somewhat irregular oval or elliptical body which is not sharply limited distally. Cells appear to become separated from its distal end and to wander peripherally along the vagus trunk. Mi-

[3] *Abhdl. Math-physischen Classe d. Königl. Sächs. Gesell. d. Wiss.* Bd. 8, Leipzig 1891.

totic figures occur frequently in the ganglion of the trunk and occasionally all along the vagus nerve.

The course and the direction of the cells migrating peripherally along the fibers of the vagi are indicated by the arrows in fig. 16. That these cells actually migrate peripherally cannot be doubted. The number of "accompanying" cells present in the vagus trunks increases rapidly until a maximum number is reached in embryos 9 or 10 mm. in length; then it decreases rapidly until the embryos

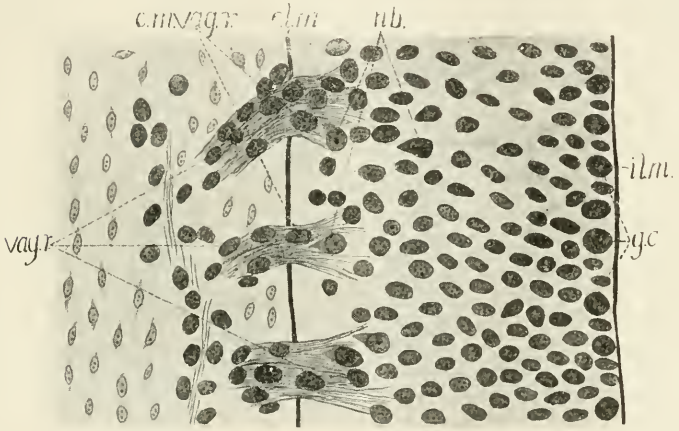

FIG. 15. Section through the rootlets of the vagus nerve in an embryo 10 mm. in length, taken at right angles to the axis of the trunk. × 270.

have attained a length of about 13 mm., when only a relatively small number of cells remains distributed along the nerve-fibers. These phenomena can be explained on no other ground. Again, the preparations studied show figures of cells escaping from the growing branches of the vagi into the anlagen of the cardiac plexus and the sympathetic plexuses in the walls of the visceral organs which are perfectly clear, and can be interpreted only to mean that these are the cells which give rise to the vagal sympathetic plexuses.

The majority of the cells migrating peripherally along the fibers of the vagi are characterized by large rounded or elongated nuclei showing a delicate chromatin structure, and very little cytoplasm.

These are obviously the "indifferent" cells of Schaper. Among these, other cells occasionally are found which are characterized by large rounded or elongated nuclei showing a well defined nucleolus and very little chromatin structure, and a larger cytoplasmic body which is usually drawn out to a point at one side (fig. 17). These cells are obviously the "neuroblasts" of Schaper. From this description it is obvious that the cells which migrate from

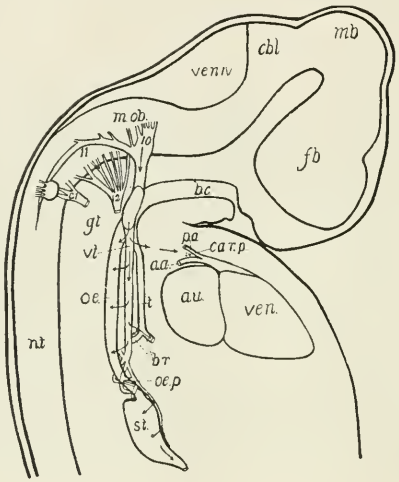

FIG. 16. Diagram designed to show the relation of the vagi to the vagal sympathetic plexuses. The arrows indicate the course and the direction of the cells migrating from the walls of the hind-brain and the vagus ganglia into the anlagen of the vagal sympathetic plexuses.
a.a., Aortic arch. *au.*, Auricle. *b.c.*, Buccal cavity. *br.*, Bronchi. *car.p.*, Anlagen of cardiac plexus. *cb.l.*, Cerebellum. *f.b.*, Fore-brain. *g.l.*, Ganglion of the trunk. *m.b.*, Mid-brain. *m.ob.*, Medulla oblongata. *n.t.*, Neural tube. *oe.*, Œsophagus. *oe.p.*, Œsophageal plexus. *p.a.*, Pulmonary artery. *st.*, Stomach. *t.*, Trachea. *ven.*, Ventricle. *ven. IV.*, Fourth ventricle. *v.t.*, Vagus trunk. *10.*, Roots of vagus nerve. 11., Roots of spinal accessory nerve. *12.*, Roots of hypoglossal nerve. *c.I.*, First cervical nerve.

the vagus ganglia and the walls of the hind-brain along the vagi are cells of the same character as those which migrate from the neural tube and the spinal ganglia along the spinal nerves.

The above observations prove conclusively that the myenteric and the submucous plexuses, the pulmonary plexuses, and the

cardiac plexus have a common origin which is distinct and separate from the origin of the sympathetic trunks. They arise from cells which have their origin in the vagus ganglia and the walls of the hind-brain. As in the case of the sympathetic trunks, however, we are not to suppose that all the cells taking part in the development of the vagal sympathetic plexuses actually migrate as such from their sources in the cerebro-spinal nervous system. Doubtless, many arise by the mitotic division of "indifferent" cells along the course of migration and in the anlagen of these plexuses. The vagus ganglia and the walls of the hind-brain, therefore, constitute a source which is sufficient to account

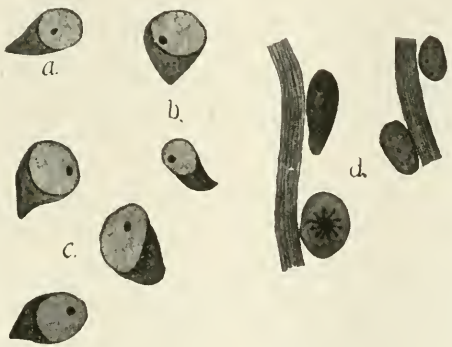

FIG. 17. Neuroblasts and indifferent cells located in the vagi and the ganglia of the trunk, × 1100.
a., Neuroblast in the vagus rootlets. *b.*, Neuroblasts in the vagus trunks. *c.*, Neuroblasts in the ganglia of the trunk. *d.*, Bundles of fibers with accompanying indifferent cells, from the vagus trunks.

for all the cells which take part in the early development of the vagal sympathetic plexuses. Migrant cells cannot be traced from the sympathetic trunks into the anlagen of these plexuses until the nerves connecting the latter with the sympathetic trunks are present. At this time the vagal sympathetic plexuses are well established, and the great majority of the cells taking part in their development are already present. We may conclude, therefore, that the nerves entering the vagal sympathetic plexuses

from the sympathetic trunks represent later connections, and play only a secondary part in their development.

These conclusions differ widely from the views hitherto generally accepted concerning the development of the cardiac plexus and the sympathetic plexuses in the walls of the visceral organs, but the facts on which they are based are perfectly clear. Furthermore, they obviate certain difficulties which arise in any attempt to derive these plexuses from the sympathetic trunks. The anlagen of the sympathetic plexuses in the walls of the visceral organs are present before any traces of the prevertebral plexuses or of cells migrating peripherally from the sympathetic trunks are found. It is obvious, therefore, that the vagal sympathetic plexuses cannot be derived from the sympathetic trunks. These findings also give the vagi an importance in the development of the sympathetic nervous system which has hitherto been unrecognized, but which is in complete harmony with other known facts.

V. DISCUSSION OF RESULTS, AND CONCLUSIONS

(*a*) *Migration of medullary cells.*—Neurological literature contains frequent allusions to the migration of medullary cells ever since the time of Balfour ('75). That pioneer among the investigators of the histogenesis of nerve-forming elements observed cells which he believed to be nervous elements, migrating from the embryonic neural tube in elasmobranchs. These observations were substantiated by Beard ('88) and Dohrn ('88, '91). Herrick ('93) observed medullary elements migrating from the motor niduli into the ventral roots of the spinal nerves in amphibians, reptiles, and mammals. Ganglion cells have also been found occasionally in the motor nerve-roots in adult animals. Such cells were observed by Freud ('78) in the ventral roots of the spinal nerves in Petromyzon, and by Schäfer ('81) and Kölliker ('94) in the ventral roots of the spinal nerves in the cat. Thompson ('87) described cells which he interpreted as degenerating ganglion cells, in the third and fourth cranial nerves in man.

More recent investigators have frequently observed migrant

medullary cells and have variously interpreted them. Harrison ('01) has shown that in the salmon the spinal ganglia arise from cells which migrate out from the dorsal region of the neural tube. He also observed medullary cells migrating into the ventral roots of the spinal nerves, and suggested the possibility that these cells may migrate peripherally into the ganglia of the sympathetic trunks and there give rise to sympathetic excitatory neurones. Bardeen ('03) observed that in mammalian embryos a certain number of cells migrate from the neural tube and the spinal ganglia along the fibers of the spinal nerves. He suggested that these cells take part in the development of the neurilemma. He believes, however, with Vignal ('83) and Gurwitsch ('00), that in mammals the neurilemma is derived largely from the mesoderm. Neal ('03) described medullary cells in the ventral roots of the spinal nerves in Squalus acanthias, and expressed the opinion that they take part in the development of the neurilemma. Carpenter ('06) has shown that in embryos of the chick medullary cells which he recognizes as the "indifferent" cells of Schaper, migrate from the walls of the mid-brain along the fibers of the abducent and the oculomotor nerves. According to Carpenter most of these cells become distributed along the nerve-trunks and may be recognized as the cells which give rise to the neurilemma. In the oculomotor nerve, however, some of these "indifferent" cells migrate farther peripherally and give rise to neurones in the ciliary ganglion. Carpenter and Main ('07) are of the opinion that some of the medullary cells which they observed migrating into the ventral roots of the spinal nerves in embryos of the pig become cells of the neurilemma and there subserve a supporting function similar to that of the neuroglia cells in the central nervous system. Cajal ('08) described elements which he recognizes as nerve cells in the bipolar phase, in the ventral roots of the spinal nerves and certain of the cranial nerves in the chick.

Although the advocates of the theory of local differentiation and the multicellular nature of nerve-fibers reject the theory of the migration of nervous elements, the results of recent researches are so convincing that we must accept the migration of medullary

cells as a fact. The present series of observations shows, moreover that the migration of medullary elements plays a far more important rôle in the development of the peripheral nervous system than has hitherto been admitted. Direct observations have shown that medullary cells migrate into the ventral roots of the spinal nerves and into the roots of several of the cranial nerves. The present observations have further shown that such cells migrate also into the dorsal roots of the spinal nerves and into the roots of the vagus and the spinal accessory nerves. I have also observed medullary cells migrating into the semilunar ganglia. Furthermore, it has been shown that some of the cells which migrate peripherally from the neural tube and the cerebrospinal ganglia give rise to the sympathetic nervous system.

(*b*) *The neurilemma.*—An extended discussion of the development of the neurilemma is beyond the scope of this paper. Inasmuch, however, as the histogenesis of the neurilemma is so intimately related to the histogenesis of the sympathetic neurones, its origin may be considered briefly at this point. As the "indifferent" cells migrate peripherally from the neural tube and the spinal ganglia, they migrate not only into the anlagen of the sympathetic trunks but also along the growing fibers beyond the origin of the communicating rami. These cells as well as the cells which, as has been shown, remain distributed along the nerve-trunks after migration has ceased, obviously take part in the development of the neurilemma. They cannot be accounted for in any other way.

Not a few of the more recent investigators, including the advocates of the theory of local differentiation and the multicellular nature of nerve-fibers, are of the opinion that the neurilemma is of ectodermal origin. We agree with the advocates of local differentiation on this point, but we must disagree with them as to the manner in which the cells giving rise to the neurilemma arise and are distributed along the nerve-fibers. It is significant that Kölliker ('05), though formerly of the opinion that the neurilemma is of mesodermal origin, came to the conclusion, in his last research, that some of the cells which wander out from the spinal ganglia give rise to the neurilemma of the sensory fibers, and that the neurilemma is everywhere of ectodermal origin. Carpenter ('06) has

shown that migrant medullary cells actually develop into cells of the neurilemma in the abducent and the oculomotor nerves in the chick.

Proof of the medullary and the ganglionic origin of the cells giving rise to the neurilemma was difficult only until it could be demonstrated that cells actually migrate peripherally from the neural tube and the cerebro-spinal ganglia along both the spinal and the cranial nerves. The present series of observations presents conclusive evidence on this point. We may here repeat what has already been stated with regard to the cells taking part in the development of the sympathetic system. We are not to suppose that all the cells taking part in the development of the neurilemma actually migrate as such from the neural tube and the cerebro-spinal ganglia. Doubtless, many arise by the mitotic division of "indifferent" cells along the course of migration. According to this interpretation, the cells of the neurilemma are homologous with the neuroglia cells in the central nervous system.

(*c*) *Sympathetic excitatory and sympathetic sensory neurones.*— The problem of the histogenetic relationships of the sympathetic excitatory and the sympathetic sensory neurones presents peculiar difficulties. The presence of sympathetic sensory neurones in the sympathetic trunks and prevertebral plexuses has not been demonstrated. Froriep, like Langley, Kölliker, and P. Schultz, denies the existence of sympathetic sensory neurones entirely. There can be little doubt, however, that sympathetic sensory neurones are present in the sympathetic plexuses in the walls of the digestive tube. According to Bayliss and Starling ('99), the peristaltic contractions of the small intestine are true coördinated reflexes carried out by the local nervous mechanism (myenteric plexus). The later experimental work of Cannon ('06) and of Auer ('10) lends support to this view by showing that the peristaltic contractions of the stomach and the intestine may be carried on more or less regularly for a considerable length of time after both the vagi and the splanchnic nerves have been severed. These phenomena seem to indicate the existence of true sensory neurones in the sympathetic plexuses in the walls of the digestive tube.

Froriep's conclusion that the sympathetic neurones have their origin in the ventral half of the neural tube and migrate out along the fibers of the ventral roots of the spinal nerves is probably correct with regard to the neurones in the sympathetic trunks and the prevertebral plexuses. I have shown, however, that the vagal sympathetic plexuses arise from cells which migrate peripherally along the fibers of the vagi. If, as experimental evidence indicates, so ne of these plexuses contain sensory neurones, it is probable that these arise from cells which migrate from the vagus ganglia. While it is impossible by direct observation to trace either sympathetic excitatory or sympathetic sensory elements back to their specific source in the cerebro-spinal nervous system, the facts at our command warrant the conclusion that the sympathetic excitatory neurones arise from cells which migrate from the neural tube along the fibers of the motor nerve roots, while the sympathetic sensory neurones, wherever such neurones exist, arise from cells which migrate from the cerebro-spinal ganglia.

The nervous elements in the neural crest obviously have the same origin as those which remain within the neural tube; they are the descendents of the "germinal" cells of His. Retzius has shown that in amphioxus sensory neurones are found lying near the internal limiting membrane lining the slit-like central canal, some of which send their dendrites out to the skin. In the fishes also a relatively large number of cells remaining within the neural tube give rise to sensory fibers which run to the skin. In embryos of the salmon, according to Harrison, cells become separated from the neural tube and, migrating ventrally, give rise to the spinal ganglia. In embryos of the pig, as already indicated, medullary cells migrate from the dorsal region of the neural tube into the dorsal nerve-roots. All these facts suggest that the cerebro-spinal ganglia have arisen from cells which originally lay within the neural tube, and indicate the common origin of all sensory and motor neurones.

The orientation of the cells in the neural tube, during the period of migration, is such that the cells which wander into the dorsal nerve-roots seem to have their origin in the dorsal part of the neural tube, while those which migrate into the ventral nerve-

roots wander out from the ventral zone and from the region in which later the lateral horn of the gray matter arises. This also is in full accord with the conditions in the adult nervous system. The neurones in the cerebro-spinal ganglia, as far as is known, are sensory in character, while the cells whose axones constitute the fibers of the motor nerve-roots are located in the ventral part of the neural tube. Furthermore, the investigations of Kohnstamm ('00) render the existence of efferent fibers in the dorsal nerve-roots of the higher vertebrates extremely doubtful. Inasmuch as nervous elements which have the capacity to develop into neurones migrate peripherally along both the sensory and the motor nerve-roots, we are driven to the conclusion that the sympathetic excitatory elements migrate from the neural tube along the fibers of the motor nerve roots, while the sympathetic sensory neurones, wherever such neurones exist, arise from cells which wander out from the cerebro-spinal ganglia. This interpretation makes the sympathetic neurones entirely homologous with the efferent and the afferent components of the other functional divisions of the peripheral nervous system.

(*d*) *A wider application of Schaper's conception.*—As has been shown in the preceding pages, the cells which migrate peripherally from the neural tube and the cerebro-spinal ganglia have a common origin; they are the descendants of the "germinal" cells of His; viz., the "indifferent" cells and the "neuroblasts" of Schaper. Therefore, Schaper's conception of the developmental relationships of the neurones and the supporting elements in the central nervous system may be extended to the sympathetic neurones and the cells of the neurilemma.

(*e*) *The relation of the sympathetic to the central nervous system.*—In the light of the present investigation, the sympathetic system bears a direct genetic relationship to the central nervous system. The cells giving rise to the sympathetic trunks, and the prevertebral plexuses migrate peripherally along the spinal nerves, while those giving rise to the vagal sympathetic plexuses migrate peripherally along the vagi. The cells giving rise to the sympathetic neurones, however, all have the same genetic relationships; they are the descendants of the "germinal" cells of His.

Therefore, the sympathetic neurones are homologous with the neurones in the central nervous system.

The sympathetic system is not a nervous mechanism separate from the central nervous system, but the nervous system is a unit of which the sympathetic system is a part homologous with the other functional divisions. It may be looked upon as an accession to the nervous system which has arisen comparatively late in the evolution of vertebrates, in response to an increasing demand for a nervous mechanism of a lower order, which might assume the direct control of the purely vegetative functions.

(*f*) *Functional relations.*—The reader will, undoubtedly ask what bearing the facts set forth in the preceding pages may have on physiological and psychological problems involving the sympathetic system. This question we cannot hope to answer at present. We may, however, offer a few suggestions which have presented themselves during the progress of this investigation.

Our knowledge concerning the functional relations and the physiological activities of the sympathetic system is very limited. Nor could we hope for much progress in this direction as long as the developmental relationships of the sympathetic to the central nervous system were not definitely known. The fact that the sympathetic system is homologous with the other functional divisions of the nervous system lends a new aspect to the entire problem. The fact, however, that the vagal sympathetic plexuses have their origin in the hind-brain and the vagus ganglia will probably be of even greater physiological and psychological importance. This fact indicates a close relationship between the lower centers of the brain and the innervation of the heart and the visceral organs. The suggestion is here ventured that in this relationship will probably be found the basis of certain physiological and psychological problems involving the digestive functions and the action of the heart, which have hitherto been obscure.

Here is a field for investigation which challenges the attention of both the student of physiology and the student of psychology. It is beset with the greatest difficulties, but promises to be fruitful of the most far-reaching results.

VI. SUMMARY

1. The sympathetic trunks arise as a pair of cell-columns lying along the sides of the dorsal surface of the aorta. In the early stages, medullary cells migrate from the neural tube into the dorsal and the ventral nerve-roots. The cells which migrate into the ventral nerve-roots, with similar cells which wander down from the spinal ganglia, migrate peripherally along the spinal nerves. Some of these cells deviate from the course of the spinal nerves and, migrating along the paths of the communicating rami, give rise to the sympathetic trunks. These findings differ materially from those of the earlier investigators. They agree essentially with the findings of Froriep.

2. The prevertebral plexuses arise as cell-aggregates lying along the ventro-lateral aspects of the aorta in the posterior region of the body. They are derived directly from the sympathetic trunks.

3. The cardiac plexus and the sympathetic plexuses in the walls of the visceral organs are not derived from the sympathetic trunks, as has hitherto been supposed, but have their origin in nervous elements which migrate from the hind-brain and the vagus ganglia along the fibers of the vagi. In view of the relation of these plexuses to the vagi, the author has chosen to designate them as "vagal sympathetic" plexuses. These findings give the vagi an importance in the development of the sympathetic system which has hitherto been unrecognized.

4. The cells migrating peripherally from the cerebro-spinal system along the spinal nerves and the vagi are the descendants of the "germinal" cells of His; viz., the "indifferent" cells and the "neuroblasts" of Schaper. Therefore they are homologous with the cells giving rise to the neurones and the supporting elements in the central nervous system.

5. The cells migrating peripherally along the spinal nerves and the vagi do not all take part in the development of the sympathetic system. Some become distributed along the nerve-fibers and give rise to the neurilemma. Therefore, the cells of

the neurilemma are homologous with the neuroglia cells in the central nervous system.

6. The cells taking part in the development of the sympathetic nervous system and the neurilemma do not all actually migrate as such from their sources in the cerebro-spinal system. Doubtless, many arise by the mitotic division of "indifferent" cells along the course of migration.

7. The existence of sympathetic sensory neurones in the sympathetic trunks and the prevertebral plexuses has not been demonstrated. Experimental evidence, however, indicates the presence of sympathetic sensory neurones in the sympathetic plexuses in the walls of the digestive tube. While it is impossible, by direct observation, to trace either sympathetic excitatory or sympathetic sensory elements back to their specific source in the cerebro-spinal nervous system, indirect embryological and anatomical evidence warrants the conclusion that the sympathetic excitatory neurones arise from cells which migrate from the neural tube along the fibers of the motor nerve-roots, while the sympathetic sensory neurones, wherever such neurones exist, arise from cells which migrate from the cerebro-spinal ganglia. This interpretation makes the sympathetic neurones homologous with the afferent and the efferent components of the other functional divisions of the peripheral nervous system.

8. Inasmuch as the cells migrating peripherally from the cerebro-spinal nervous system are the "indifferent" cells and the "neuroblasts" of Schaper, Schaper's conception of the developmental relations of the neurones and the supporting elements in the central nervous system, may be extended to the sympathetic neurones and the cells of the neurilemma.

9. The nervous system is a unit of which the sympathetic system is a part homologous with the other functional divisions. The sympathetic system may be looked upon as an accession to the nervous system, which has arisen comparatively late in the evolution of vertebrates in response to the conditions of the vegetative life.

10. The fact that the sympathetic system is homologous with the other functional divisions of the nervous system lends a new aspect to the problems involving its functional relations. The fact that the vagal sympathetic plexuses have their origin in the hind-brain and the vagus ganglia will, doubtless, have an important bearing on certain physiological and psychological problems involving the heart action and the digestive functions.

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THE CONTROL OF PHOTOTACTIC REACTIONS IN HYALELLA BY CHEMICALS

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Loeb[1] in a brief preliminary paper has shown that specimens of Gammarus pulex, which are normally negatively phototactic, may be made positive if they are placed in certain chemicals of the proper degree of concentration. For example, positive phototaxis was produced when the amphipods were placed in solutions of hydrochloric, oxalic or acetic acid of about m/500. Loeb obtained similar results with a 2½m solution of ethyl alcohol, a m/6 solution of ether, and a m/25 solution of ammonium chloride, but a m/10 solution of boracic acid failed to produce such an effect. My own experiments were performed on another amphipod, Hyalella knickerbockeri, in the endeavor to ascertain if Loeb's results would hold true in this species, and to test the effect of various chemicals in different concentrations. The results obtained were similar to those of Loeb, but it was found that Hyalellas were made positive by boracic acid if they were dropped into a saturated solution. Tartaric acid produces no change in their reaction.

My results with salts were quite parallel to those of Loeb; I found some ammonium salts to make them decidedly positively phototactic; some potassium salts made them weakly positive; potassium bromide and potassium iodide made them strongly positive. Potassium chloride and potassium chlorate produced no marked change in their phototactic response, nor did any of the sodium salts, or magnesium sulphate. I tried several alkalies, but here, as with the salts, there seemed to be no relation be-

[1]Loeb, J. The Control of Heliotropic Reactions in Fresh-water Crustacean, by Chemicals, especially CO_2. *University of California Publications.* Physiology vol. 2, p. 1. 1904.

tween the chemicals used and the reactions. Some potassium salts produced a change in the phototaxis of the animals, other potassium salts did not; some acids produced a change in the phototaxis, others did not. It is the same with the alkalies; ammonium hydroxide causes all the animals to become positive immediately when they are dropped into a solution of .0075 per cent, but when the animals are put into a solution of potassium hydroxide, or sodium hydroxide, or potassium carbonate of any concentration which will not kill them outright, there is no change in their phototactic response; they still remain negative. Loeb[2] claims that in all probability light produces chemical changes in the eye or skin of the animals, and that these changes are responsible for the phototactic reactions. If this be true, it might seem not improbable that some definite relation would be found between the classes of chemicals employed and the reaction, but such experiments as have thus far been made tend to prove that no such relation exists.

In endeavoring to test more thoroughly the effects of chemicals upon phototaxis, experiments were conducted in the dark room, the source of light being an electric tungsten bulb of 350 candle-meters intensity. Seven chemicals were used, namely, ethyl alcohol, ammonium hydroxide, and hydrochloric, nitric, acetic, picric, and chromic acids. I first determined the lowest per cent solution of each of these chemicals which would cause a reversal in the phototactic reaction of the Hyalellas; in other words, the least per cent solution which would make them positive when they were dropped directly into it. I found that ethyl alcohol of .074 per cent, ammonium hydroxide of .0075 per cent, hydrochloric acid of .0067 per cent, nitric acid of .0053 per cent, acetic acid of .01 per cent, picric acid of .0053 per cent, and chromic acid of .0046 per cent would produce this result. In each of these cases, all or nearly all the animals would be in the positive end of the dish making frantic efforts to get nearer the source of light. My next experiment was to place several of the animals in an oblong glass dish, four inches long and one and one-half inches

[2]Loeb, J. Comparative Physiology of the Brain and Comparative Psychology. *New York*, 1900, and *l.c.*

wide, containing twenty-five cubic centimeters of distilled water. Very slowly and gradually the concentration of the solution was increased by adding constant small amounts of a given chemical at intervals of five to fifteen minutes. A careful record was kept of the number of animals positive and the number negative at certain concentrations throughout the experiments. In every case the light was moved and the reaction of the animals tested from each end of the dish; that is, when the animals had oriented themselves in one end of the dish, the light was then transposed to that end and the reaction tested again. The results were surprising; in each case the amphipods remained decidedly negatively phototactic, even though the concentration was carried far beyond the point at which they became positive when dropped directly into the solution. The concentration in each case was increased to the point where the majority of the animals died; nevertheless they were negative, and decidedly so, throughout the experiment until death occurred. Thus, when the concentration is *gradually* increased, the Hyalellas were negative in .64 per cent solution of ethyl alcohol, .05 per cent solution of ammonium hydroxide, .022 per cent hydrochloric acid, .05 per cent nitric acid, .43 per cent acetic acid, .029 per cent picric acid, and 0.22 per cent chromic acid. The results will appear more evident by a study of the accompanying table. In this table the first column of figures indicates the per cent solution; following each of these figures, to the right, is indicated the number of animals which were positive and the number negative at each reading of a given chemical at a concentration given in the first column. The line drawn across each column indicates the point at which the animals were positively phototactic when dropped directly into the solution. Where the sum of the positive and negative specimens is not equal to the sum of the positive and negative used at the beginning of the experiment, it indicates that a number of animals died from the effect of the chemical.

It is evident that it is not chemical change in the tissues which caused the reversal of reaction, for, if it were, it would be impossible to increase the concentration and still have the animals

TABLE OF PHOTOTACTIC REACTION IN HYALELLA.

PER CENT SOLUTION USED	NITRIC ACID	ACETIC ACID	PICRIC ACID	CHROMIC ACID	HYDRO-CHLORIC ACID	AMMONI-UM HY-DROXIDE	ALCOHOL
	No. + −	No. + −	No. + −	No. + −	No. + −	No. + −	No. + −
.0000	0 21	0 17	0 20	0 18	1 20	0 20	0 20
.0008	0 21	0 17	0 20	0 18	0 21	0 20	1 19
.0016	0 21	1 16	0 20	0 18	0 21	0 20	0 20
.0023	0 21	0 17	0 20	1 17	0 21	0 20	0 20
.0031	0 21	0 17	0 20	0 18	0 21	0 20	0 20
.0038	0 21	0 17	0 20	0 18	0 21	0 20	0 20
.0046	0 21	0 17	0 20	1 17	0 21	0 20	0 20
.0053	0 21	0 17	0 20	2 15	0 21	0 20	0 20
.0060	0 21	0 17	1 19	1 13	0 21	0 20	0 20
.0067	0 21	0 17	4 16	0 14	0 21	0 20	0 20
.0075	0 21	0 17	2 16	0 9	0 21	0 20	0 20
.0100	0 21	0 17	1 14	0 9	0 21	1 19	0 20
.015	0 21	0 17	1 12	0 7	0 14	0 19	0 20
.022	0 21	0 17	1 11	0 4	0 3	1 15	0 20
.029	0 21	1 16	2 8			1 12	0 20
.036	0 19	0 17				0 12	0 20
.043	0 13	0 17				0 7	0 20
.050	0 4	1 16				0 5	0 20
.060		0 17					0 20
.067		0 17					0 20
.074		0 17					1 19
.100		0 17					1 19
.150		1 16					0 20
.220		0 17					0 20
.290		0 17					1 18
.360		0 14					0 19
.430		0 6					0 18
.500							0 18
.570							0 18
.640							0 18

remain negative; even though the increase be gradual, the chemical change in the eye or skin of the animal would take place as readily when the necessary concentration was reached as when the animal was dropped directly into the concentrated chemical. Moreover, the duration of immerison in the chemical is greater when the concentration is gradually increased; this would tend to produce a more complete chemical change in the animal than when it is dropped momentarily into the chemical. It is probable, therefore, that these various changes of reaction are due, not to chemical changes in the eyes or skin of the animal, but to a sudden stimulation or shock to the nervous system.

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THE DEVELOPMENT OF THE HYPOGLOSSAL GANGLIA OF PIG EMBRYOS

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EIGHT FIGURES

The use of dissected pig embryos for classwork in embryology suggested itself to the writer some years ago. Upon trial it was found that very instructive preparations could be made and with much greater ease than might be expected. It is difficult for students to grasp the relations of developing organs as seen in sections and a dissected embryo showing the primitive organs in position is very helpful in remedying this evil. It is the intention of the author to present, in a future paper, the results of his work along these lines, with directions for dissection and figures of the more instructive preparations. The form and relations of the various organs may be seen as accurately as in reconstructions made from serial sections by experts. There is this disadvantage in dissection, that some of the finer details of structure may be lost. For research it commends itself as a check to errors which may occur in making reconstructions; for it enables one in a short time to study a considerable number of embryos.

The nervous system lends itself most easily to dissection. The mesenchyma crumbles away from the more tenuous nervous tissue of suitably prepared embryos, making it possible to lay bare the entire nervous system of pigs varying in length from 6 to 20 mm. The smaller embryos are more easily dissected, as no cartilage or bone is encountered.

My dissections brought out some points in connection with the cerebral nerves which have not hitherto been cleared up, and my

purpose in the present paper is to describe the rudimentary ganglia which occur between the first cervical nerve and the vagus, ganglia which I shall refer to as the hypoglossal ganglia because of their undoubted connection with this nerve. My descriptions will necessarily deal with the development of the last four cranial nerves, the glosso-pharyngeal, the vagus, the spinal accessory, and the hypoglossal.

LITERATURE

The occurrence of hypoglossal ganglia was first described by Froriep ('82) in the sheep and the ox. He traced the development of a single ganglion, anterior to the first cervical, which it resembled in form, though smaller in size. The single distal root of this ganglion joined the most caudad root of the hypoglossal nerve. Anterior to this hypoglossal ganglion the neural crest was undifferentiated. Froriep and Beck ('95) found a precervical ganglion present in the adult throughout those groups of mammals in which the first cervical ganglion was well developed.

Martin ('91), investigating cat embryos, found five ganglionic masses posterior to the jugular ganglion, and five roots of origin for the hypoglossal nerve. He concludes, therefore, that these ganglia are the dorsal ganglia of the hypoglossal, though he gives no figures in support of his view.

Lewis ('03) in his excellent paper on the anatomy of a 12 mm. pig found extending caudad from the jugular ganglion "a beaded commissure ending in a small knob. In the track of the commissure, but separated from it, is an irregular ganglionic mass. After another interval there appears a small fragment, then follows the first cervical ganglion." In one case he found a small fiber bundle connecting the irregular ganglionic mass (Froriep's ganglion) with the hypoglossal nerve, but considers its "relation with the commissure" as "far more striking than its resemblance with a spinal ganglion." He finds the ganglion "connected with the commissure in pigs of 17 mm." In a dissected pig of the same length "the hypoglossal ganglion appeared as a detached part of

the ganglionic chain running forward to the vagus. This commissure could not be subdivided into definite ganglia; it was characterized by irregular swellings and spurs."

Streeter ('04) in tracing the development of the peripheral nerves in human embryos finds a ganglionic crest extending from the first cervical to the superior ganglion of the glossopharyngeal and partly ensheathing the fibers of the spinal accessory nerve. In embryos 10 to 13 mm. long the neural crest becomes differentiated into four or five rather diffuse cell masses. Froriep's ganglion resembles the others, being irregular in form and without roots. The hypoglossal nerve originates as four or five parallel roots. There is no correspondence between these and the rudimentary ganglia, nor are the ganglia segmentally arranged. He considers the three or four anterior cell masses as cerebral ganglia and "not to be confused with the precervical ganglion of Froriep."

MATERIALS AND METHODS

The number and length of the embryos dissected are given in the following table:

STAGE	LENGTH	LENGTH OF EXAMPLE	NUMBER DISSECTED
I	5–7 mm.	6 mm.	5
II	8–10 mm.	8.5–9 mm.	4
III	12–14 mm.	13–13.5 mm.	10
IV	17–20 mm.	17–18 mm.	10
V	28–30 mm.	28 mm.	4
VI	41–50 mm.		2
	Total		35

All drawings were made with the aid of an Abbe camera lucida and a Zeiss a* objective. The embryos were fixed in Zenker's fluid and the dissections were first stained, cleared in creosote and drawn as transparent objects. It was thus possible to locate microscopic cell masses and trace the course of very small fiber bundles. The dissection was then transferred to alcohol and examined as

an opaque object by reflected light to obtain the contour of the different structures. By making several dissections of the same stage I believe that the finer structures were more accurately and completely reproduced than could be done by serial reconstructions.

DESCRIPTION OF DISSECTIONS

Stage 1. 6 mm. In this embryo (fig. 1) the ganglia were connected from the glossopharyngeal (*IX*) back to the caudal region

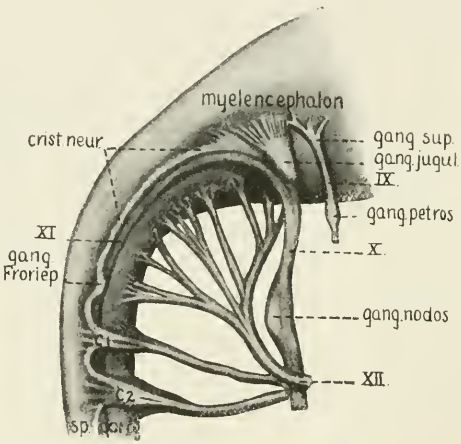

Fig. 1. Dissection of a 6 mm. pig showing in the hypoglossal region the undifferentiated neural crest. See explanation of figures on page 282.

by continuous bands or loops of cells, undifferentiated portions of the neural crest. The ventral roots of the spinal nerves (C_1 C_2) are large, but the dorsal ganglia show little differentiation into fibers, though short distal and proximal roots are present. The hypoglossal originates as five or six parallel roots, resembling those of the spinal series but uniting, in the later embryos of this stage, to form a common trunk (*XII*). The spinal accessory (*XI*), as an arched bundle of fibers, could be traced from the fourth cervical ganglion cephalad to the vagus. Dorsal to the accessory and partly ensheathing it is a flattened band of cells, the

neural crest (*crist. neur.*), extending forward to the jugular ganglion of the vagus (*gang. jugul.*). Opposite the posterior root of the hypoglossal a marked ventral loop and thickening in the crest (*gang. Froriep*) shows the position of Froriep's ganglion. Anteriorly the crest of cells is broader and a few short proximal rootlets are present. A depression separates it more or less completely from the cells of the jugular ganglion which is flattened and diffuse with 8–10 short proximal roots. The glossopharyngeal is short and its superior ganglion is joined to the jugular ganglion by a small cord of cells.

The remarkable features at this stage are then: the early development of the spinal roots; the resemblance of the hypoglossal to a series of ventral spinal roots; the existence of a nearly undifferentiated neural crest between the jugular and the first cervical ganglion.

Stage 2. 8.5–9 mm. In embryos of this stage (figs. 2 and 3), the roots of the spinal nerves are longer and more fibers are developed. The first cervical ganglion is distinctly double in fig. 2. It is still connected with the second cervical ganglion by a loop of cells. The neural crest between the first cervical and the jugular ganglia shows the most marked change. The slight enlargement opposite the posterior root of the hypoglossal which we saw in the first stage has now grown to be a spindle-shaped mass of cells (*gang. Froriep*) with two proximal roots and a distal bundle of fibers which extends to the root of the hypoglossal. This ganglion (Froriep's) is still connected with cellular loops (*nod.*) of the neural crest, but in this respect it does not differ from the cervical and sacral ganglia of this stage. It strongly resembles one of the two cell masses composing the first cervical ganglion. Anterior to it is a smaller mass of cells (*gang. hypogl.*) from which a proximal root is developing. This is the "terminal knob" of the "commissure" figured by Lewis in the 12 mm. pig (1903, pl. I). Anteriorly the crest shows five diffuse irregular cell masses which become gradually larger toward the jugular ganglion with indications of proximal roots. The jugular ganglion is of more definite form and is pointed ventrally. Dorsal

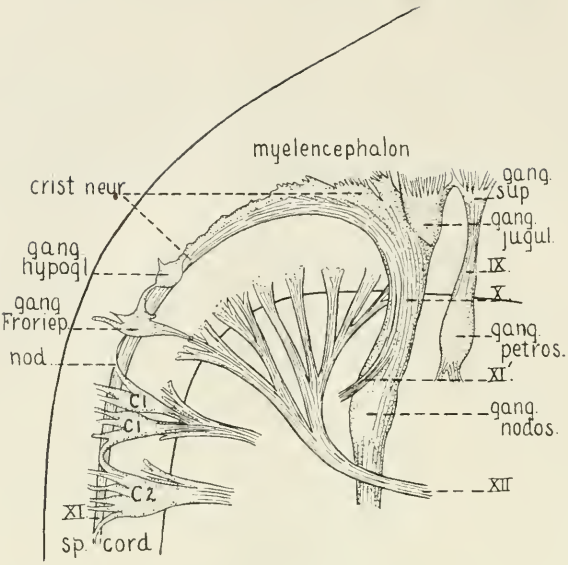

FIG. 2. Dissection of an 8.5 mm. pig showing Froriep's ganglion with a distal root to the hypoglossal nerve, and a double cervical ganglion.

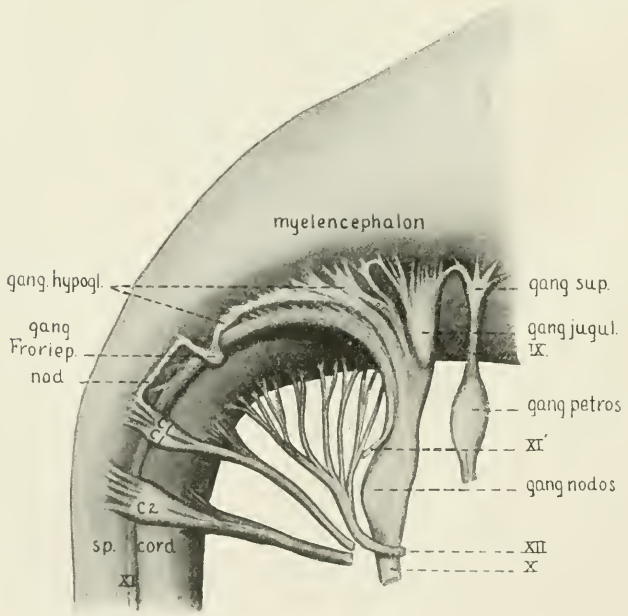

FIG. 3. Dissection of a 9 mm. pig showing an early stage in the differentiation of the neural crest to form the hypoglossal ganglia.

to the ganglion nodosum of the vagus (*gang. nodos.*) a small bundle of fibers is given off to form the peripheral portion of the spinal accessory (*XI'*).

Two embryos of 8.5 mm. showed the structure of fig. 2. In two others of 9 mm. no distal root has developed from Froriep's

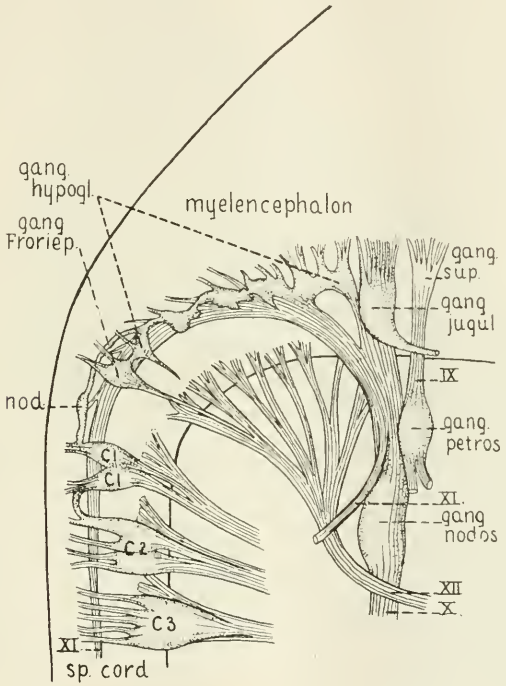

Fig. 4. Dissection of a 13 mm. pig to show a series of hypoglossal ganglia and two double cervical ganglia.

ganglion (fig. 3). The first cervical is not distinctly double and shows no connection with the second cervical ganglion. The proximal roots are longer, however, and the neural crest near the jugular ganglion is better differentiated. Two flat distal strands of mixed cells and fibers pass down parallel to the spinal accessory fibers and enter the vagus.

This stage brings out three important points: (1) the first cervical ganglion frequently originates as a double structure; (2)

Froriep's ganglion sends fibers to the hypoglossal at an earlier stage than has been described by other investigators; (3) its resemblance to one of the divisions of the first cervical ganglion is marked.

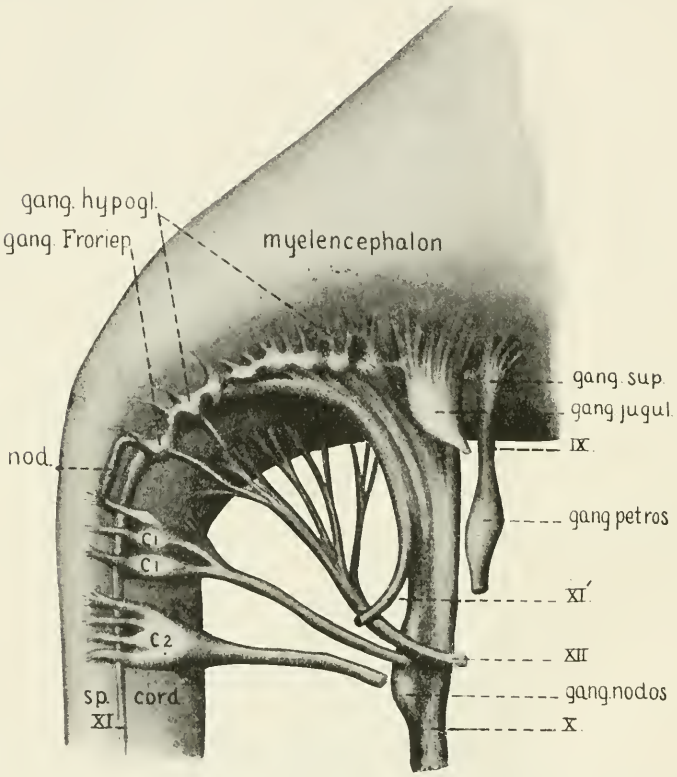

Fig. 5. Dissection of a 13 mm. pig showing a series of eight hypoglossal ganglia and the persistence of the neural crest from the superior ganglion to the first cervical.

Stage 3. 13 mm. This embryo, of about the same age as that studied by Lewis, is characterized by a further elongation of the distal and proximal roots and by a greater differentiation of the neural crest anterior to the first cervical nerve. Five of the ten embryos dissected belonged to the types shown in fig. 4 and fig. 5. Here we see a cord of cells passing cephalad from the first cervical ganglion. In fig. 5 it joins Froriep's ganglion. In fig. 4

it passes under its proximal roots. Froriep's ganglion possesses now one, now two proximal roots, with a distal bundle of fibers entering the posterior root of the hypoglossal nerve, and it is united to a more irregular ganglionic mass by a strand of cells which varies in size in different embryos. This second ganglion also shows one or two proximal roots, and a distal root is in evidence. A third cell mass more cephalad shows a proximal root, is pointed at its distal end and projects ventrad to the spinal accessory. Anterior to these three occurs a series of five cell masses, more diffuse, more closely united and elongate in the antero-posterior line. A small strand of cells unites the more cephalad of these to the jugular ganglion, and this in turn is connected with the superior ganglion of the glossopharyngeal. Each of these cell masses possesses from two to four proximal roots, and two pairs of the adjacent roots are joined by cellular loops. Distal roots either join the spinal accessory or form part of flattened bundles which pass to the trunk of the vagus.

In three of these five embryos the first cervical ganglion was distinctly double as in figs. 4 and 5. In the other two it gave evidence of a double origin. An interesting point was the difference in structure exhibited on the right and left sides of the same embryo. For example, in two other cases Froriep's ganglion was well developed with a distal hypoglossal root on the right side, small and without hypoglossal root on the left side. In two cases no hypoglossal root was found on either side, a condition similar to that figured by Lewis, who, however, found a small distal fiber bundle in a second embryo.

To sum up: The 13 mm. embryo shows (1) the neural crest anterior to the first cervical ganglion differentiated into about eight ganglionic masses; (2) the two posterior of these send roots to the hypoglossal in the majority of cases, and the condition figured by Lewis is apparently the exception rather than the rule; (3) a persistence of cellular cords still unites the various links in this chain of ganglia with each other and with the first cervical and jugular ganglia; (4) the first cervical ganglion is often double in structure, and all the spinal ganglia are elongate and unlike the rounded nodules figured by Lewis; (5) in the ganglionic chain

it is not possible to distinguish an anterior cranial series belonging to the vagus complex, and a pre-cervical group of spinal ganglia as maintained by Streeter ('04); (6) the connection between the chain of ganglia and the vagus is not as marked in most cases as Lewis figures, and when well developed represents a persistence

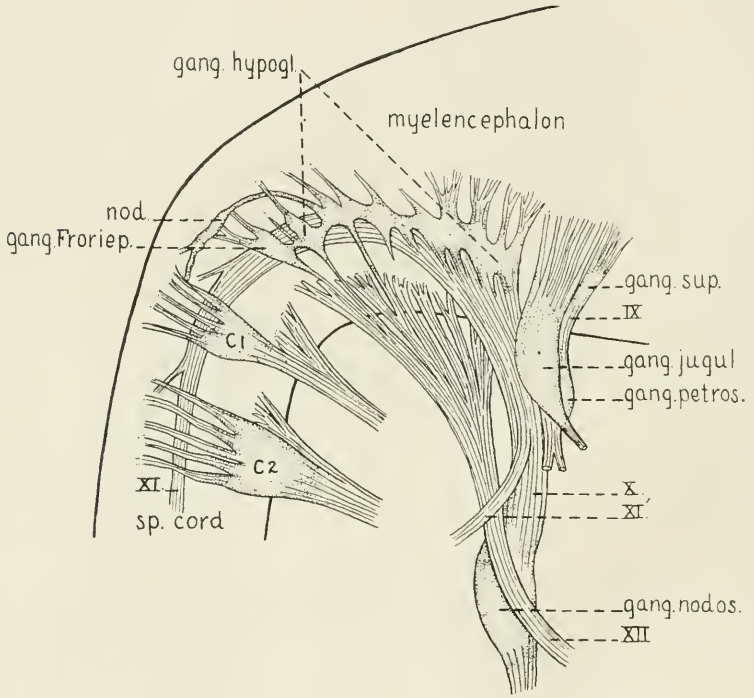

Fig. 6. Dissection of a 17 mm. pig showing three distal roots passing from the hypoglossal ganglia to the ventral roots of the hypoglossal nerve and a fourth incomplete root.

of the ganglionic crest—a persistence common also to the spinal ganglia; (7) the spinal accessory root could be traced back to the sixth cervical ganglion.

Stage 4. 17–18 mm. Further elongation of the nerve roots is accompanied by a differentiation of the hypoglossal ganglia greater than at any other stage (figs. 6 and 7). The ganglia are relatively smaller but a continuous cord of cells may still be traced

from the first cervical to the jugular ganglion. In fig. 7, the first cervical ganglion is double. Froriep's ganglion is independent of the rudimentary crest and shows two proximal roots, one connecting with a small lateral spur. The distal root is large and

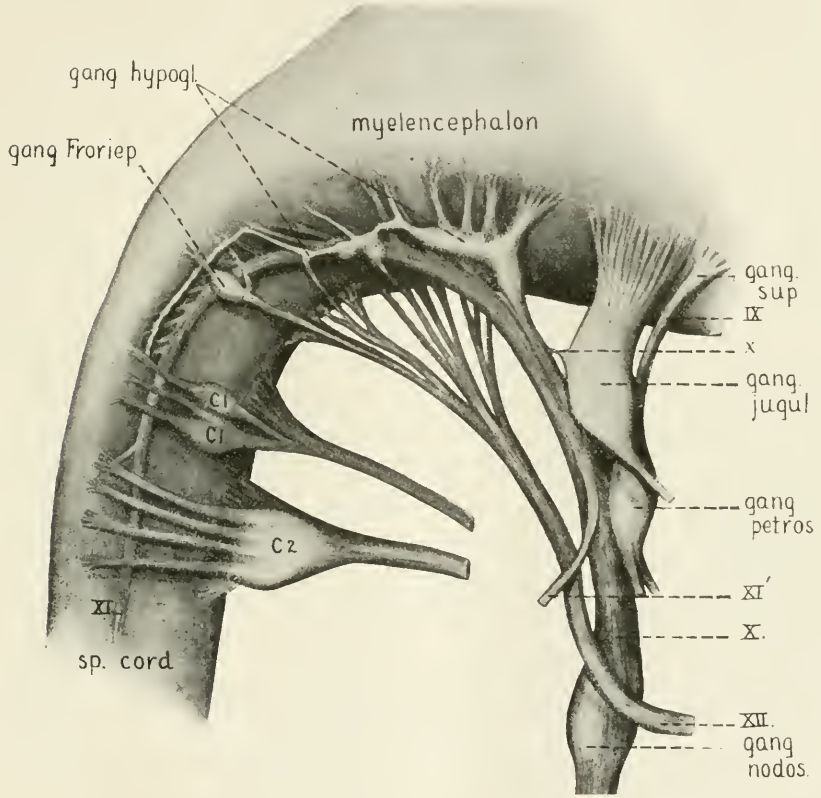

FIG. 7. Dissection of an 18 mm. pig with three distal roots passing from the hypoglossal ganglia to the ventral roots of the hypoglossal nerve. The first cervical ganglion is double and the neural crest is persistent from the first cervical to the jugular ganglion.

its fibers join the caudal root of the hypoglossal some distance ventral to the spinal cord. Next anterior are three connected masses of ganglion cells which increase in size cephalad. Two proximal and two distal roots are seen. The distal roots are extremely small and it is doubtful whether their fibers enter the

hypoglossal. Anterior to these three ganglionic masses is a large ganglion elongate in the line of the ganglionic crest. Five groups of proximal roots arise from the myelencephalon and distal roots join the spinal accessory. The anterior of these is the largest. Soon after it unites with the spinal accessory a short very slender cord of cells and fibers passes over to the jugular ganglion (fig. 7, *x*). This is the only connection between the hypoglossal ganglia and the jugular ganglion of the vagus.

The hypoglossal ganglia are best developed in the 17 mm. embryo shown in fig. 6. In this case three distal roots join the hypoglossal from as many ganglia, and a fourth distal spur is present. The first cervical ganglion is only partially divided. Comparing the hypoglossal ganglia with one division of the first cervical ganglion as seen in fig. 4, the resemblance is plain.

From ten dissections at this stage we would note the following points: (1) The hypoglossal ganglia here reach their highest differentiation; (2) in every case Froriep's ganglion was present with a well developed hypoglossal root,—in three cases two such hypoglossal roots were present, in one case the ganglion was forked and in one case (fig. 6) there was evidence of four hypoglossal ganglia with distal roots; (3) this stage proves that the connection between the jugular ganglion and the hypoglossal ganglion is of little importance other than showing that both are derived from a common neural crest; (4) as observed in preceding stages, there is great variation in the hypoglossal ganglia of different individuals, and on the two sides of the same embryo; no two were exactly alike; (5) the root of the spinal accessory could be traced back to the eighth cervical ganglion.

Stage 5. 28–30 mm. In succeeding stages the hypoglossal ganglia show retrogressive changes as to structure and relative size. Fig. 8 shows the persistence of a single hypoglossal ganglion (Froriep's) posteriorly. Anteriorly three closely connected ganglia are seen, the more posterior sending a spur backward, which ends abruptly. This condition was found in two cases. In one case a double hypoglossal ganglion was present, and in one case a small spurred fragment occupied a position near the

middle of the hypoglossal chain, that is, about midway between Froriep's ganglion and the jugular ganglion.

Stage 6. 41–50 mm. Two dissections showed conditions similar to the preceding. A double Froriep's ganglion was found

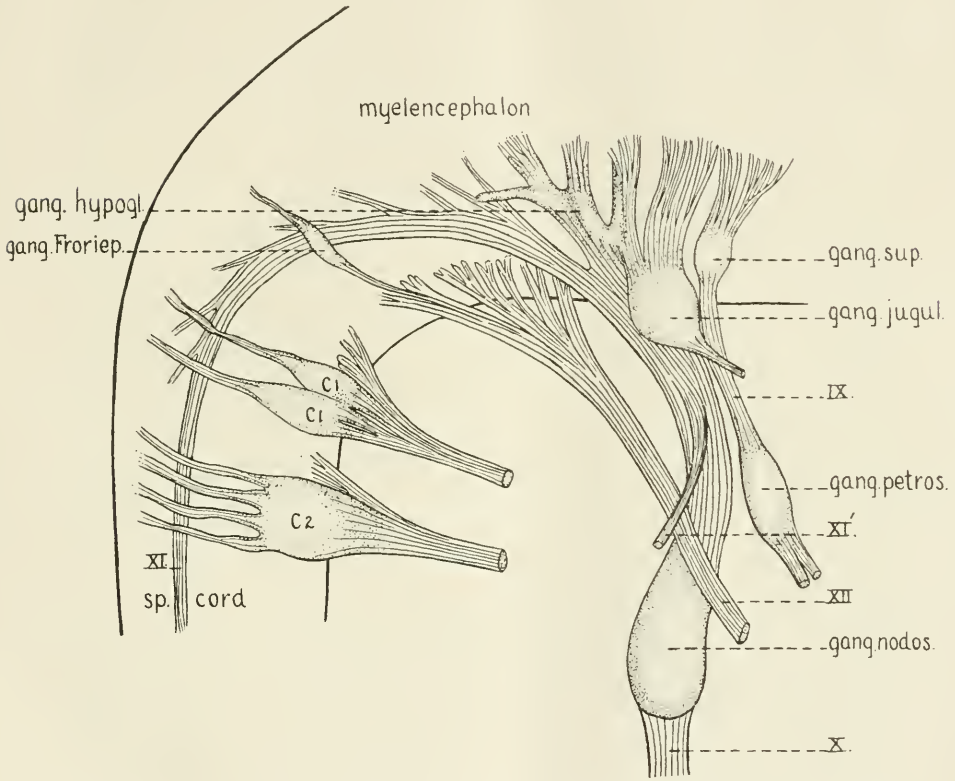

FIG. 8. Dissection of a 28 mm. pig showing Froriep's ganglion and a distal root to the hypoglossal nerve. The middle portion of the series of hypoglossal ganglia, present in earlier stages, has disappeared.

in one case relatively smaller than in the embryo of 30 mm. Its arrested development was shown by its unchanged position and small size. It lies still partly dorsal to the spinal accessory, while the cervical ganglia have shifted ventrad owing to the elongation of their roots, and to their own growth.

THEORETICAL DISCUSSION

It seems evident from the different dissections we have made that the hypoglossal nerve first develops as several ventral spinal roots (5 to 6 in number) which arise independently, lie parallel to each other and are in series with spinal nerves. Later these independent roots unite to form the trunk of the hypoglossal nerve. Comparing the earlier stages with the later, it would seem that the more anterior roots atrophy and this is in harmony with the observations of Bremer ('08).

Allowing that the hypoglossal is a composite of ventral spinal roots, then we should expect to find their ganglia, if present, between the first cervical and jugular ganglia. We do find a chain of ganglia occupying this very position, but they are rudimentary, appear late and soon show retrogressive changes. They arise from the same neural crest as do the spinal ganglia and root ganglia of the vagus and glossopharyngeal. They form a continuous series, but show variations in form characteristic of all rudimentary structures. As far as their early development is concerned they cannot be divided into a pre-cervical and a cerebral group, nor is there an overlapping of spinal and cerebral ganglia, according to the theory of Froriep.

Objections have been raised as to whether these ganglia were really homologous with spinal ganglia. The points made have been: (1) Difference in form, these rudiments rarely resembling spinal ganglia; (2) their frequent connection with the vagus rather than with the hypoglossal; (3) their lack of segmental arrangement; (4) their many variations and irregularities of form.

As to their form, in the sheep Froriep found it similar to that of the cervical ganglia. In the pig where they are best developed they are usually spindle-shaped, but broader forms, resembling spinal ganglia, have occasionally been observed. In many mammals too, including man, the first cervical ganglion loses its typical form and may become vestigial. In the pig the first cervical is smaller than the other spinal ganglia and develops

later. I have frequently found it double, consisting of two spindle-shaped masses of cells. Other spinal ganglia show the same condition. The first cervical ganglion possesses always several proximal roots (4–5) and the distal root arises as *two distinct bundles*. Froriep's ganglion never shows more than two proximal roots, generally only one, and never more than one distal root. As these ganglia are also spindle-shaped I would regard them as not homologous with a spinal ganglion, but as comparable to one of the spindle-shaped divisions of such a cervical ganglion as seen in fig. 4. Two of the rudimentary hypoglossal ganglia with their two distal roots would be exactly homologous with a single spinal ganglion. The separation of the two parts of the ganglion could be accounted for as due to their arrested development; as they do not appear until late and the pre-cervical region grows more rapidly, the two masses of cells representing a ganglion would be separated to a greater or less extent. At any rate, we find that the first cervical ganglion is frequently divided in the same way, sometimes the second cervical, and the same thing may occur throughout the spinal series. The irregularities of structure and constant variation which we find in the hypoglossal ganglia is merely typical of all rudimentary structures.

Lewis has objected that the connection of the hypoglossal ganglia with the vagus is more marked than their relation to the hypoglossal. He figures the hypoglossal ganglia (his "beaded commissure") as continuous with the jugular ganglion. I have shown that the direct connection with the jugular ganglion is only important as showing their common development from the neural crest. Occasionally this connection entirely disappears and it is to be compared to the loops of cells which may persist between the proximal roots of two adjacent spinal ganglia. Furthermore as many as three ganglia may be connected by distal roots with the roots of the hypoglossal. It is my opinion that the anterior ganglia of this series originally related to the hypoglossal, have become connected with the vagus complex, just as in man some fibers from the first cervical ganglion have joined the spinal accessory.

The present lack of segmental arrangement displayed by these ganglia does not preclude their metameric origin. Their development begins considerably later than that of the spinal nerves, and the rapid growth of the region they occupy, before they make their appearance, may cause them to shift their positions with relation to their myotomes. They certainly appear in regular series and their early development is similar to that of the spinal ganglia.

As to the number of dorsal ganglia represented in the hypoglossal series, no absolute statement can be made. The evidence of comparative anatomy goes to show that four or five spinal nerves have been added to the cranial series as a result of the union with the cranium of a corresponding number of vertebrae. Meeks ('09) finds in an Acanthias embryo three rudimentary spinal ganglia located between the vagus and the first spinal nerve, the ganglion of which would correspond to Froriep's ganglion in mammals. According to this evidence, four dorsal ganglia have become rudimentary structures in mammals and the corresponding ventral roots have united to form the hypoglossal trunk. Regarding each pair of the eight ganglionic nodules found in the 13 mm. embryo as homologous to a single spinal ganglion, then we would have the same number of ganglia, four, represented between the first cervical and the jugular. The more anterior roots of the hypoglossus, which are found in the early embryos but disappear in the later stages, represent ventral roots of the vagus and glossopharyngeal according to the observations of Bremer ('08).

CONCLUSIONS

1. The jugular and superior ganglia of the vagus and glossopharyngeal nerves, the hypoglossal ganglia and ganglia of the spinal nerves arise in the pig embryo from a continuous neural crest, as observed by Streeter in human embryos.

2. The hypoglossal ganglia are retarded in their development, but appear in embryos of 13 mm. as a series of eight connected cell masses of nearly equal size.

3. According to their development, the hypoglossal ganglia can be divided only artificially into a cephalic cerebral group and a caudal pre-cervical group.

4. The first cervical and other spinal ganglia are often of double origin, composed of two spindle-shaped masses, and generally possess two distal roots.

5. The spindle-shaped ganglion of Froriep with its single distal root would therefore represent but one half of a spinal ganglion.

6. The degree of development of the hypoglossal ganglia varies in different embryos; in the same embryo the right side may be better developed than the left, and vice versa. This is good evidence of their rudimentary or vestigial character.

7. One, frequently two or three, and in one case four hypoglossal ganglia possessed single distal roots and the fibers of three of these joined the hypoglossal nerve.

8. The connection of the hypoglossal ganglia with each other and with the jugular ganglion represents a persistence of the neural crest. It is similar to the connections which were found persisting between the roots of the spinal ganglia.

9. If we consider a pair of hypoglossal ganglia as the equivalent of a single spinal ganglion, four such ganglia would be represented in pig embryos, between the jugular and the first cervical.

10. The hypoglossal trunk develops as five or six separate ventral roots, at first parallel and independent, later uniting to form a single nerve.

11. The hypoglossal ganglia reach their maximum development in embryos 17–20 mm. long, then retrograde, though ganglia at both ends of the series may persist in the adult.

12. The spinal accessory nerve develops very early, being well formed in the youngest embryos examined (5 mm. long). As development proceeds the fibers of the spinal accessory root may be recognized farther and farther caudad. In a pig of 17 mm. a few accessory fibers were traced to a point opposite the eighth cervical ganglion.

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EXPLANATION OF FIGURES

All drawings were made with the aid of a camera lucida and have been reduced to a common magnification of about 25 diameters. The figures show in surface view the right side of the myelencephalon and spinal cord from a point anterior to the origin of the glossopharyngeal nerve to a point just caudad to the first, second or third cervical ganglion. The following abbreviations have been employed:

C1, C2, first and second cervical ganglia; **crist. neur.,** neural crest; **gang. Froriep,** Froriep's ganglion; **gang. hypogl.,** hypoglossal ganglia; **gang. jugul.,** jugular ganglion; **gang. nodos.,** ganglion nodosum; **gang. petros.,** ganglion petrosum; **gang. sup.,** superior ganglion; **nod.,** persisting cellular nodules of neural crest; **sp. cord,** spinal cord; **IX, X, XI, XIII,** glossopharyngeal, vagus, spinal accessory and hypoglossal nerves; **XI′,** peripheral portion of spinal accessory nerve.

THE DEVELOPMENT OF THE SYMPATHETIC NERVOUS SYSTEM IN BIRDS[1]

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TEN FIGURES

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INTRODUCTION

The present investigation of the development of the sympathetic nervous system in birds has grown out of an investigation of the development of the sympathetic nervous system in mammals. It was undertaken in order to further exact knowledge concerning the development of the sympathetic nervous system, to extend

[1] From the Laboratories of Animal Biology of the State University of Iowa. Prof. Gilbert L. Houser, Director.

the writer's observations on the histogenesis of the sympathetic nervous system in mammals, and to point out certain morphogenetic differences in the development of the sympathetic system in birds and in mammals, with a view to their phylogenetic significance.

Birds and mammals have become specialized along divergent lines. Their special habits of life have brought about modification in the course of ontogeny as well as in adult structure. The sympathetic nervous system, which is concerned primarily with the control of the purely vegetative functions, has not escaped the modifying influence of specialized habits. It is hoped, therefore, that a more exact knowledge of the development of the sympathetic nervous system in birds may throw some new light on the problems involving the structural and the functional relationships of the sympathetic system to the central nervous system.

Inasmuch as the literature bearing on the development of the sympathetic nervous system has been reviewed by the writer in a recent paper,[2] only such references will be made to the literature in this paper as seem to be necessary.

The observations set forth in the following pages are based on embryos of the chick. The embryos were fixed in chrom-aceto-formaldehyde. The sections were cut to a thickness of 10 micra and stained by the iron-hæmatoxylin method. This method, as indicated in the earlier paper, was found best adapted for purposes of this research.

OBSERVATIONS

1. Sympathetic trunks

(*a.*) *Introductory.*—His, Jr. ('97) called attention to the fact that in the chick two pairs of sympathetic trunks arise in the course of ontogeny. These he has designated as the "primary" and the "secondary" sympathetic trunks. According to his observations, the primary sympathetic trunks arise about the close of the third

[2] The development of the sympathetic nervous system in mammals. *Journal of Comparative Neurology and Psychology*, vol. 20, no. 3.

day of incubation, as a pair of cell-columns lying along the sides of the dorsal surface of the aorta. About the beginning of the sixth day, the anlagen of the secondary sympathetic trunks arise as cell-aggregates situated just median to the ventral roots of the spinal nerves. These cell-aggregates are at first independent of each other, but become united later by longitudinal commissures. Between the fourth and the eighth day of incubation, the primary sympathetic trunks disappear, except in the most anterior region, becoming resolved into the ganglia and nerves constituting the prevertebral and the peripheral sympathetic plexuses. According

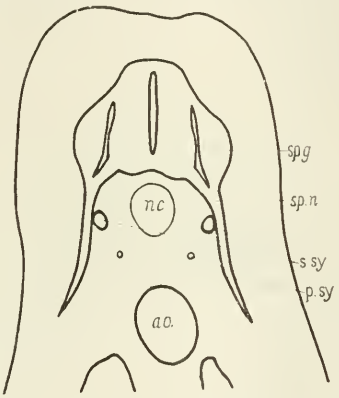


Fig. 1. Diagramatic transverse section through the thoracic region of an embryo of the chick (130 hours incubation). *ao.*, aorta; *nc.*, notochord; *p.sy.*, primary sympathetic trunks; *sp.g.*, spinal ganglion; *sp.n.*, spinal nerve; *s.sy.*, secondary sympathetic trunks.

to His, Jr., the cells giving rise to both the primary and the secondary sympathetic trunks are derived exclusively from the spinal ganglia.

My observations on the development of the sympathetic trunks in the chick do not differ essentially from those of His, Jr., except in one particular. I find that the cells giving rise to the primary and the secondary sympathetic trunks in the chick, like the cells giving rise to the sympathetic trunks in mammals, are not derived exclusively from the spinal ganglia, as His, Jr., believes them to be, but that they have their origin, wholly or in part, in the neural tube.

Figure 1 has been introduced to show the relative positions of the primary and the secondary sympathetic trunks in an embryo of the chick in the 130-hour stage.

(*b.*) *Primary sympathetic trunks.*—The primary sympathetic trunks arise about the beginning of the fourth day of incubation, as cell-aggregates lying along the sides of the aorta and along the dorsal surfaces of the carotid arteries. At the close of the fourth day (96-hour stage), these cell-aggregates have assumed the appearance of loosely aggregated cell-columns (fig. 2, A, *p. sy.*). Well marked ganglionic enlargements do not occur, but the cell-columns are not of uniform diameter. In the posterior region, the anlagen of the primary sympathetic trunks arise a little later than in the anterior region, and remain less sharply limited. They are, at this stage, not directly connected with the spinal nerves. In the thoracic region where the spinal nerves are best developed, they extend peripherally a little beyond the level of the aorta. At a point a little above the level of the aorta, cells deviate from the course of the spinal nerves and wander through the mesenchyme, either singly or in small groups, toward the sides of the aorta (fig. 2, A and B, *i. c. c. r.*) where they become aggregated to give rise to the anlagen of the primary sympathetic trunks.

During the course of the fifth day of incubation, the primary sympathetic trunks become more conspicuous. They move dorsally and recede a short distance from the walls of the aorta until at the close of the fifth day (120-hour stage) they appear as conspicuous cell-columns lying along the dorso-lateral aspects of the aorta a short distance from its surface (fig. 2, C, *p. sy.*). The primary sympathetic trunks are now sharply defined in the anterior region and are connected with the spinal nerves by distinct cellular tracts. In the posterior region, the cell-aggregates are still loosely scattered along the sides of the aorta and the cellular tracts connecting them with the spinal nerves are less distinct. The primary sympathetic trunks have now reached their maximum development. During the course of the sixth day, they decrease materially in size until at the close of the sixth day (144-hour-stage) they have almost disappeared. Their complete dis-

appearance occurs first in the thoracic region, while the last remnants may be observed in the anterior cervical region.

(*c.*) *Secondary sympathetic trunks.*—The anlagen of the secondary sympathetic trunks arise about the beginning of the sixth day (120-hour stage), as ganglionic enlargements on the median sides of the spinal nerves at the point of origin of the communicating

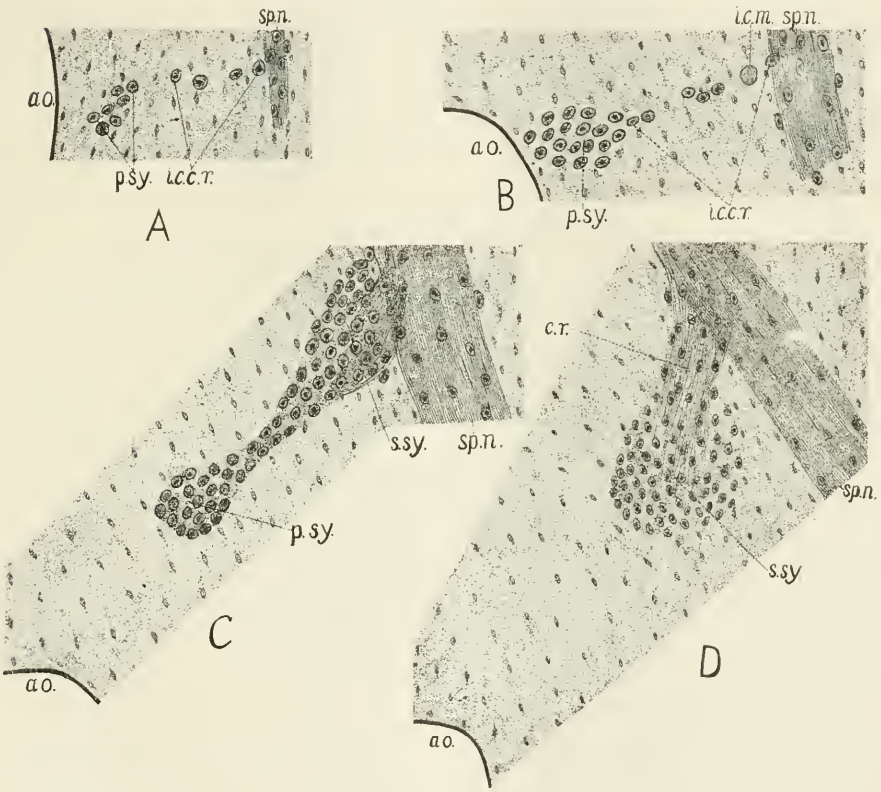


Fig. 2. Transverse sections showing successive stages in the development of the primary and the secondary sympathetic trunks in the chick. A., primary sympathetic trunk (96 hours incubation), × 200. B., Primary sympathetic trunk (105 hours incubation), × 200. C., Primary and secondary sympathetic trunks (120 hours incubation), × 200. D., Secondary sympathetic trunk (144 hours incubation), × 100. *ao.*, aorta; *c.r.*, communicating ramus; *i.c.c.r.*, cells migrating from spinal nerve to primary sympathetic trunk; *i.c.m.*, indifferent cell undergoing mitosis; *p.sy.*, primary sympathetic trunk; *sp.n.*, spinal nerve; *s.sy.*, secondary sympathetic trunk.

rami (fig. 2, C, *s. sy.*). These ganglionic enlargements are at first independent of each other, but become united later by longitudinal commissures. Like the anlagen of the primary sympathetic trunks, the anlagen of the secondary sympathetic trunks appear earliest in the thoracic region and latest in the sacral region. At the beginning of the sixth day there are as yet no traces of the anlagen of the secondary sympathetic trunks in the posterior half of the body. During the course of the sixth day, the secondary sympathetic trunks become larger and more conspicuous, while the primary sympathetic trunks become correspondingly smaller. The former, being located at the point of origin of the communicating rami, are connected with the latter by the cellular tracts which connect them with the spinal nerves (fig. 2, C).

As the communicating rami become fibrous, the anlagen of the secondary sympathetic trunks become removed a short distance from the spinal nerves. In the cervical and the thoracic region they are removed to the ends of the short communicating rami (fig. 2, D, *s. sy.*). In the posterior region of the body, the fibers of the communicating rami extend beyond the anlagen of the secondary sympathetic trunks. At the close of the sixth day, they may be traced through the cell-aggregates still remaining scattered along the sides of the aorta, into the anlagen of the prevertebral plexuses.

In the posterior region of the body, the distinction between the primary and the secondary sympathetic trunks is never well marked. Cells gradually become aggregated in the proximal part of the communicating rami to give rise to the secondary sympathetic trunks, while the cells constituting the primary sympathetic trunks migrate ventrally into the anlagen of the prevertebral plexuses. After the sixth day, the secondary sympathetic trunks become more distinct throughout their entire length, as the ganglionic enlargements become connected by the fibers of the longitudinal commissures.

(*d.*) *Histogenesis.*—As already indicated, the sympathetic trunks arise from cells which migrate peripherally from the cerebrospinal nervous system along the spinal nerves. As soon as fibers

appear in the ventral roots of the spinal nerves (72-hour stage) cells may be traced from the motor niduli, across the marginal veil, into the proximal part of the ventral nerve-roots. Medullary cells become aggregated in the proximal part of the ventral

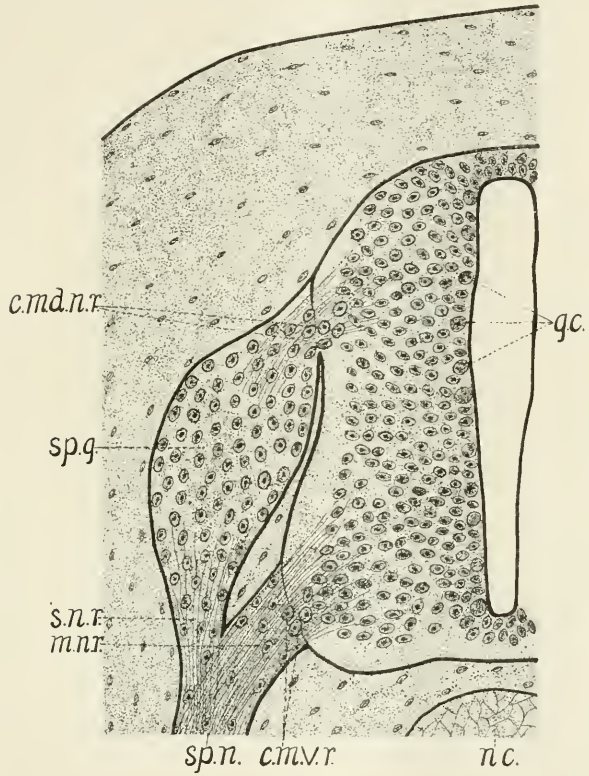


Fig. 3. Transverse section of the neural tube and the spinal ganglion of an embryo of the chick (105 hours incubation), × 190. *c.m.d.n.r.*, cells migrating into dorsal nerve-root; *c.m.v.r.*, cells migrating into ventral nerve-root; *g.c.*, germinal cells of His; *m.n.r.*, motor nerve-root; *nc.*, notochord; *sn.r.*, sensory nerve-root; *sp.g.*, spinal ganglion; *sp.n.*, spinal nerve.

nerve-roots and soon appear to migrate peripherally along the nerve-fibers. While the spinal ganglia are becoming differentiated from the neural crest, cells apparently having their origin in the neural tube wander out into the spinal ganglia. Evidence of this

process may be observed as early as the 64-hour stage. During the fourth and the fifth day, after the spinal ganglia have become well differentiated, a few cells may be observed migrating from the dorsal part of the neural tube into the dorsal nerve-roots (fig. 3, *c. m. d. n. r.*). It is probable that cells do not migrate from the dorsal part of the neural tube in any considerable numbers after the spinal ganglia have become differentiated. Cell migration into the dorsal nerve-roots is probably only a transient process which takes part in the development of the spinal ganglia.

As the cells in the ventral nerve-roots migrate peripherally, they mingle with similar cells which wander down from the spinal ganglia. As there is no recognizable difference between the cells which wander out from the spinal ganglia and those which migrate peripherally along the ventral nerve-roots, it is impossible to distinguish between the cells from these two sources after they have passed beyond the point of union of the sensory and the motor nerve-roots. As these cells migrate peripherally along the spinal nerve-trunks, some of them deviate from the course of the spinal nerves and migrate toward the sides of the aorta where they become aggregated to give rise to the primary sympathetic trunks. As migration proceeds, the cells which deviate from the course of the spinal nerves no longer migrate into the primary sympathetic trunks, but become aggregated at the median sides of the spinal nerves to form the ganglionic enlargements which constitute the anlagen of the secondary or permanent sympathetic trunks.

His, Jr., has expressed the opinion that the elements composing the primary sympathetic trunks are resolved into the ganglia and nerves of the prevertebral and the peripheral sympathetic plexuses. In view of the comparatively enormous development of the prevertebral plexuses and of the ganglion of Remak in birds, this is obviously the fate of the elements composing the primary sympathetic trunks in the posterior region of the body. There is no evidence, however, of the peripheral migration of cells from the primary sympathetic trunks in the anterior region. While there may be some migration posteriorly along the primary sympathetic trunks, it is more probable that most of the elements composing

these trunks in the anterior region of the body are withdrawn into the anlagen of the secondary or permanent sympathetic trunks along the cellular tracts connecting the former with the latter. The last remnants of the primary sympathetic trunks in the anterior cervical region, as His, Jr., has suggested, probably atrophy.

The period of incubation being comparatively shorter in birds than in mammals, cell migration takes place much more rapidly. It is at its height in the chick during the fourth and the fifth day of incubation. During this time breaches occur frequently in the external limiting membrane of the neural tube just opposite

Fig. 4. Neuroblasts drawn with the aid of the camera lucida, × 825. *a.*, in ventral nerve-root inside external limiting membrane (105 hours incubation); *b.*, in ventral nerve-root outside external limiting membrane (105 hours incubation); *c.*, in spinal nerve (105 hours incubation); *d.*, in communicating ramus (105 hours incubation); *e.*, in ventral nerve-root (96 hours incubation); *f.*, in spinal nerve (96 hours incubation).

the motor niduli, and medullary cells may be traced without difficulty from the motor niduli into the proximal part of the ventral nerve-roots (fig. 3, *c. m. v. r.*). Numerous accompanying cells are present in the spinal nerve-trunks as far as the latter may be traced. At the close of the sixth day, the number of cells present in the spinal nerves has materially decreased. While cells are still moving peripherally along the spinal nerves, it is probable that migration from the neural tube and the spinal ganglia has practically ceased.

The great majority of the cells migrating peripherally along the spinal nerves are characterized by very little cytoplasm, and

by large rounded or elongated nuclei usually having their chromatin aggregated into one or two dense masses. These are obviously the "indifferent" cells of Schaper. Among these are found a few cells which are characterized by large rounded or elongated nuclei showing one or two dense masses of chromatin, and a larger cytoplasmic body which is usually drawn out to a point at one side. Fig. 4 shows several of these cells drawn with the aid of the camera lucida. These are obviously the "neuroblasts" of Schaper. The majority of the cells present in the mantle layer in the neural tube answer to the descriptions given above for the two types of cells migrating peripherally along the spinal nerves. There can be no doubt, therefore, that the cells accompanying the fibers of the spinal nerves have the same histogenetic relationships as the cells which give rise to the neurones and the neuroglia cells in the central nervous system. They are all the descendants of the "germinal" cells (Keimzellen) of His.

These observations are in full accord with the writer's observations on mammalian embryos. There is a marked difference, however, in the chromatin structure of the embryonic medullary cells in birds and in mammals. In mammalian embryos, the migrant medullary cells are usually quite readily recognized by the chromatin structure of their nuclei. They also usually take a slightly deeper stain than the cells of the surrounding mesenchyme. In birds, the chromatin structure of the embryonic medullary cells differs very little from the chromatin structure of the typical mesenchyme cells. Nor are they as distinctly separated from the cells of the surrounding mesenchyme by differential stains as is the case in mammals. Although the difficulties in technique are greater in the chick than in mammalian embryos, there can be no doubt that the cells accompanying the fibers of the spinal nerves are migrant medullary cells. Such cells wander out of the neural tube into the ventral nerve-roots in considerable numbers. The number of cells present in the proximal part of the spinal nerves increases rapidly until the maximum rate of migration is reached, and then decreases rapidly until migration ceases, when only a comparatively small, but fairly constant, number of cells remain distributed along the nerve-fibers. Furthermore, a few of the

cells present in the spinal nerves are obviously neuroblasts. Such cells have frequently been observed outside the neural tube and the spinal ganglia. Cajal ('08) described cells which he recognized as nerve cells in the bipolar phase, in the motor roots of the spinal nerves and in certain of the cranial nerves in the chick. These cells, he believes, correspond to the real motor cells in the neural tube. Mitotic figures occur occasionally all along the course of migration and in the sympathetic anlagen. We are not to suppose, therefore, that all the cells taking part in the development of the sympathetic trunks actually migrate as such from their sources in the cerebro-spinal nervous system. Doubtless, many arise by the mitotic division of "indifferent" cells along the course of migration.

2. Prevertebral plexuses

The prevertebral plexuses are derived directly from the primary sympathetic trunks. They arise about the middle of the fourth day (108-hour stage), as cell-aggregates lying along the ventro-lateral aspects of the aorta from the suprarenal bodies posteriorly. In this region the primary sympathetic trunks are not sharply limited ventrally. Sympathetic cells may be traced from the latter directly into the anlagen of the prevertebral plexuses. In the sacral region, the aorta is soon completely surrounded ventrally by a ring of loosely aggregated sympathetic cells (fig. 5, *hyp.*).

The cell-aggregates constituting the anlagen of the prevertebral plexuses increase very rapidly. At the close of the fourth day (120-hour stage), these plexuses have become well established. Distinct lines of cells may be traced from the primary sympathetic trunks directly into cell-aggregates of considerable size lying along the median sides of the suprarenals, and wandering sympathetic cells may be observed all along the sides of the aorta from the suprarenals posteriorly. The limits of the anlagen of the several prevertebral plexuses cannot be determined at this stage. Traces of one or the other of these plexuses are not wanting in any transverse section in this entire region.

As incubation proceeds, the prevertebral plexuses assume more definite proportions. The cells increase in number and become more closely aggregated. At the close of the sixth day (144-hour stage), nearly all the cells which were present in the primary sympathetic trunks in the posterior region of the body have wan-

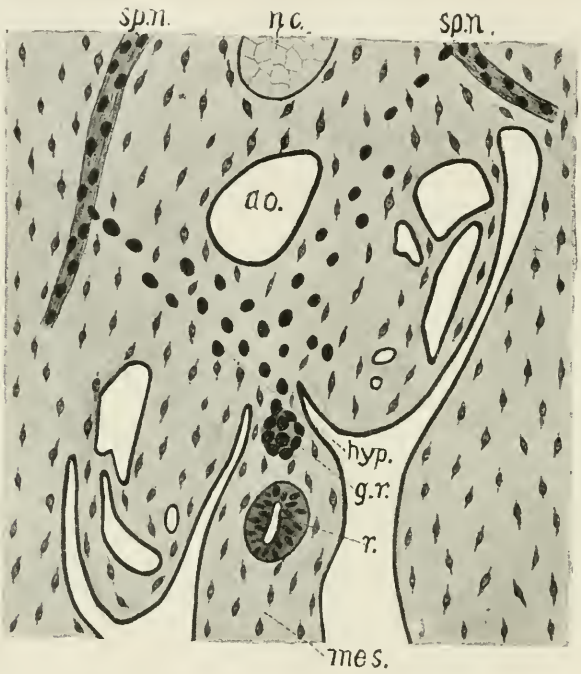


Fig. 5. Transverse section through the sacral region of an embryo of the chick (105 hours incubation), × 60. *ao.*, aorta; *g.r.*, ganglion of Remak; *hyp.*, hypogastric plexus; *mes.*, mesentery; *nc.*, notochord; *r.* rectum; *sp.n.*, spinal nerve.

dered down into the prevertebral plexuses. Neither cells nor fibers can be traced ventrally from the prevertebral plexuses as yet except in the sacral region. Here numerous cells may be traced from the hypogastric plexus directly into the ganglion of Remak.

3. *Ganglion of Remak*

The ganglion of Remak arises about the middle of the fourth day, as an oval cell-column lying in the mesentery just dorsal to the rectum (fig. 5, *g.r.*). Its greatest diameter occurs in the pos-

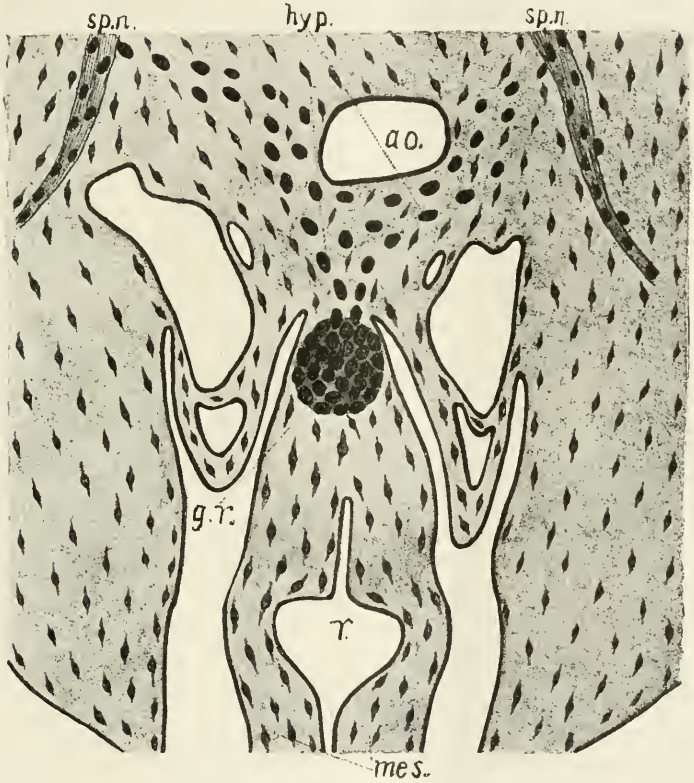


Fig. 6. Transverse section of an embryo through the sacral region of an embryo of the chick (130 hours incubation), × 110. *ao.*, aorta; *g.r.*, ganglion of Remak; *hyp.*, hypogastric plexus; *mes.*, mesentery; *r.*, rectum; *sp.n.* spinal nerve.

terior region. It increases in size very rapidly until at the close of the fifth day it has become a large and conspicuous column of closely aggregated cells, with a maximum diameter of about 85 micra (fig. 6, *g.r.*). Its diameter decreases anteriorly until it

terminates in the region of the genital ridges, in a slender cellular cord which Remak has called the intestinal nerve (Darmnerv).

This ganglion was described by Ónodi ('86) and by His, Jr. ('97), but, as far as I have been able to learn, no worker before me has traced the cells composing it to their source. My preparations show conclusively that the cells giving rise to the ganglion of Remak are derived directly from the anlagen of the hypogastric plexus. In transverse sections through the posterior sacral region, where the mesentery is broad and the rectum lies close to the anlagen of the hypogastric plexus, cells may be traced from the latter directly into the ganglion of Remak (figs. 5 and 6).

The ganglion of Remak has no counterpart in mammals. It may be of interest to note at this point that an examination of several embryos of the turtle (kindly placed at my disposal by Dr. F. A. Stromsten, of these laboratories) has shown that while there is no well defined ganglion in this type, corresponding to the ganglion of Remak, there are numerous cell-aggregates associated with the rectum, which evidently constitute the prototype of Remak's ganglion. It is probable, therefore, that this ganglion, so enormously developed in birds, is correlated with oviparous habits.

4. Vagal sympathetic plexuses

(*a.*) *Introductory.*—In an earlier paper I have shown that in mammals the sympathetic plexuses related to the vagi; viz., the cardiac plexus and the sympathetic plexuses in the walls of the visceral organs, have their origin in cells which migrate from the vagus ganglia and the walls of the hind-brain along the fibers of the vagi. I have, therefore, designated these plexuses as the "vagal sympathetic" plexuses. My observations on embryos of the chick show clearly that in birds also these plexuses have their origin in cells which migrate from the hind-brain and the vagus ganglia.

(*b.*) *Myenteric and submucous plexuses.*—In embryos of the chick in the 130-hour stage, the vagus trunks may be traced posteriorly along the walls of the œsophagus just a little below its ventral

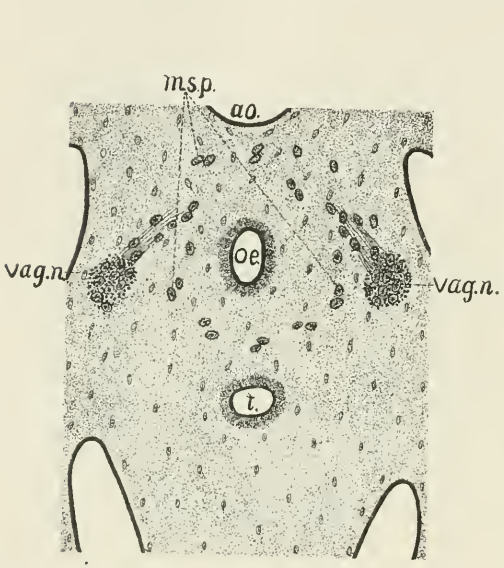
level. The ganglia of the trunk lie close to the walls of the œsophagus just distal to the origin of the trachea. The bifurcation of the trachea occurs farther anteriorly in birds than in mammals, and the bronchi are comparatively longer. Anterior to the bifurcation of the trachea, cells deviate from the course of the vagi along the fibers of their growing branches and wander into the walls of the œsophagus. These cells are so slightly differentiated at this stage that it is no longer possible to trace them after they have entered the denser tissues of the œsophageal walls. Beyond the bifurcation of the trachea, the vagus trunks bend laterally and ventrally round the bronchi and extend along the ventro-lateral aspects of the œsophagus, continually approaching each other posteriorly. At the point where the vagi begin to bend round the bronchi, each vagus trunk gives rise to a slender branch which extends posteriorly along the wall of the œsophagus between the latter and the bronchus. These branches may be traced posteriorly but for a short distance at this stage.

At the close of the sixth day, the vagi have become more conspicuous. In the anterior region, definite lines of cells may be traced from the vagus trunks into the walls of the œsophagus where they become aggregated into more or less distinct groups arranged in two broken rings (fig. 7, *m.s.p.*). Posterior to the bifurcation of the trachea, cells may be traced dorsally from the vagus trunks into the walls of the œsophagus (fig. 9, *m.s.p.*). The vagus branches lying between the walls of the œsophagus and the bronchi have become more conspicuous and may be traced posteriorly as far as the region of the lungs. Posterior to the region of the heart, the vagus trunks lie close together and apparently break up to form a plexus ventral to the œsophagus.

During the seventh and the eighth day of incubation, the sympathetic plexuses in the walls of the digestive tube become well established. Branches of the vagi may be traced into the walls of the œsophagus, and the cell-groups constituting the anlagen of the myenteric and the submucous plexuses assume a more definite arrangement.

The sources of the cells giving rise to the myenteric and the submucous plexuses in the walls of the small intestine could not

be definitely determined. In the early stages, single sympathetic cells could not be traced in the dense tissues of the walls of the digestive tube. It is difficult, therefore, to determine whether or not such cells migrate posteriorly in the walls of the digestive tube, as is the case in mammalian embryos. It is probable, however, that such is the case. On the other hand, it is probable that some of the cells which take part in the development of the myen-



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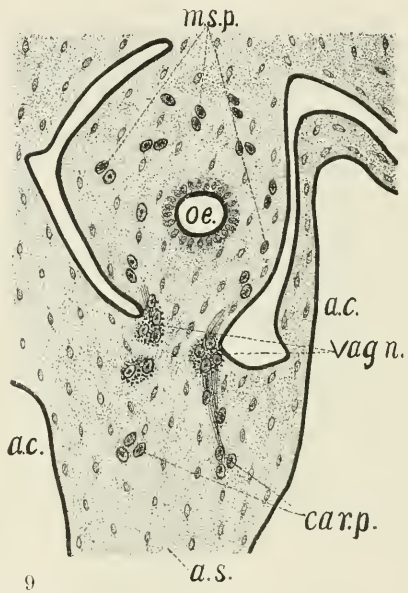

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Fig. 7. Transverse section through the œsophagus and the vagi of an embryo of the chick (144 hours incubation), × 80. *ao.*, aorta; *m.s.p.*, cells giving rise to myenteric and submucous plexuses; *oe.*, œsophagus; *t.*, trachea; *vag.n.*, vagus trunks.

Fig. 9. Transverse section through the œsophagus and the anlagen of the cardiac plexus of an embryo of the chick (144 hours incubation), × 80. *a.c.*, atrial cavity; *a.s.*, atrial septum; *car.p.*, anlagen of cardiac plexus; *m.s.p.*, cells giving rise to myenteric and submucous plexuses; *oe.*, œsophagus; *vag.n.*, vagus trunks.

teric and the submucous plexuses in the posterior region of the intestine wander out from the ganglion of Remak. There is no evidence of cells entering the sympathetic plexuses in the walls of the digestive tube from the sympathetic trunks or from the prevertebral plexuses, except through the ganglion of Remak, until

fibrous connections are established between the former and the latter. There can be little doubt, therefore, that most of the cells taking part in the development of the myenteric and the submucous plexuses in the walls of the small intestine migrate posteriorly from the anlagen of these plexuses in the anterior region of the digestive tube.

(*c.*) *Pulmonary plexuses.*—In transverse sections through the region of the lungs of embryos in the 144-hour stage, fibers may be traced laterally from the branches of the vagi lying between the œsophagus and the bronchi. Cells wander out along these

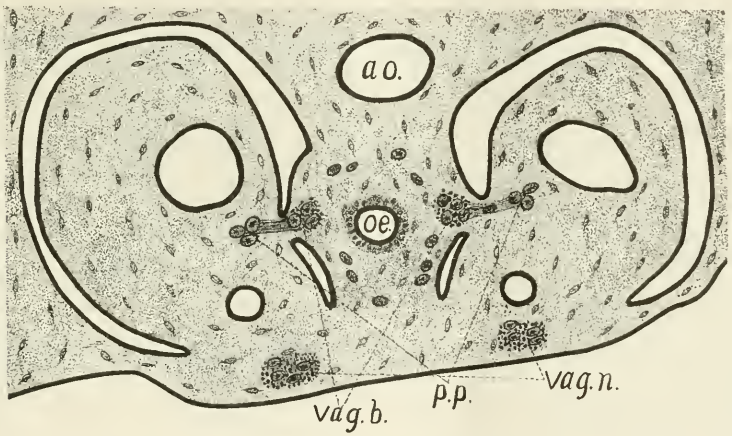

Fig. 8. Transverse section through the region of the lungs of an embryo of the chick (seventh day of incubation), × 80. *ao.*, aorta; *oe.*, œsophagus; *p.p.*, anlagen of pulmonary plexuses; *vag. b.*, branches of the vagi; *vag.n.*, vagus trunks.

fibers and become aggregated to give rise to the anlagen of the pulmonary plexuses. (fig. 8, *p.p.*).

(*d.*) *Cardiac plexus.*—In transverse sections through the region of the head in embryos in the 120-hour stage, cells may be traced ventrally from the vagi into the septum of the atria where they become aggregated into small groups which constitute the anlagen of the cardiac plexus. In later stages, these cell-groups become more conspicuous until at the close of the sixth day they appear as distinct cell-aggregates in the atrial septum (fig. 9, *car. p.*).

(*e.*) *Histogenesis.*—In sections through the head-region of embryos in the 96-hour stage, medullary cells may be traced from the walls of the hind-brain into the rootlets of the vagus and the spinal accessory nerves (fig. 10, *c. m. vag. r.*). That these cells migrate peripherally from the walls of the hind-brain in considerable numbers cannot be doubted. In many sections they may be observed pushing into the nerve-rootlets in cone-shaped heaps as the latter traverse the marginal veil. Occasionally medullary cells are observed half in and half out of the neural tube, and

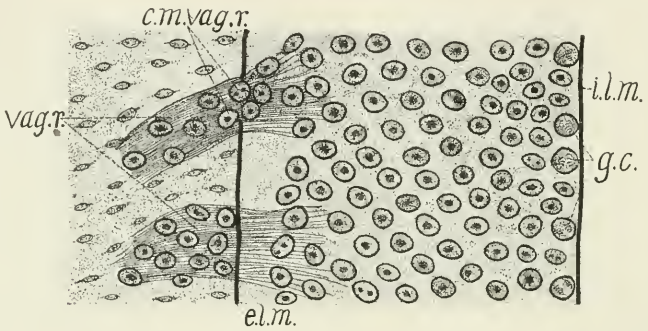

Fig. 10. Transverse section through the wall of the hind-brain of an embryo of the chick (96 hours incubation), × 550. *c.m.vag.r.*, cells migrating into roots of the vagus; *el.m.*, external limiting membrane; *g.c.*, germinal cells of His; *i.l.m.*, internal limiting membrane; *vag.r.*, roots of vagus nerve.

many are present in the nerve-rootlets just outside the external limiting membrane. With similar cells which wander out from the vagus ganglia, these cells migrate peripherally along the fibers of the vagi. As these cells migrate peripherally and the vagi give rise to fibrous branches, cells wander out from the vagus trunks and give rise to the vagal sympathetic plexuses. That such is the origin of the cardiac plexus and the sympathetic plexuses in the walls of the visceral organs in the chick cannot be doubted. The figures of cells migrating from the vagi into the anlagen of these plexuses are perfectly clear. Nor can cells be traced into these plexuses from any other

source, except possibly in the posterior region of the intestine, until fibrous connections have been established between the latter and the sympathetic trunks. By this time the great majority of the cells taking part in the development of the vagal sympathetic plexuses are already present. The connections of these plexuses with the sympathetic trunks must, therefore, be looked upon as secondary.

The period of migration of cells from the hind-brain and from the vagus ganglia along the vagi is coextensive with the period of migration of cells from the neural tube and the spinal ganglia along the spinal nerves. The cells which migrate peripherally along the vagi are cells of the same character as those which migrate peripherally along the spinal nerves; viz., they are the "indifferent" cells and the "neuroblasts" of Schaper. The cells giving rise to the vagal sympathetic plexuses, therefore, have the same histogenetic relationships as those giving rise to the sympathetic trunks. Mitotic figures occur occasionally along the vagi and in the anlagen of the vagal sympathetic plexuses. We are not to suppose, therefore, that all the cells taking part in the development of these plexuses actually migrate as such from their sources in the hind-brain and the vagus ganglia. Doubtless, many arise by the mitotic division of indifferent cells along the course of migration.

DISCUSSION OF RESULTS, AND CONCLUSIONS

The observations set forth in the preceding pages have shown that in birds the sympathetic nervous system has its origin in cells which migrate peripherally from the neural tube and the cerebro-spinal ganglia. The cells giving rise to the sympathetic trunks and the prevertebral plexuses, including the ganglion of Remak, migrate peripherally along the spinal nerves, while the cells giving rise to the vagal sympathetic plexuses migrate peripherally along the vagi. These observations agree essentially with the writer's observations on the histogenesis of the sympathetic nervous system in mammals. My observations on the histogenesis of the sympathetic system agree with the findings of Froriep ('07)

in embryos of Torpedo and of the rabbit only in regard to the sympathetic trunks and the prevertebral plexuses. Froriep succeeded in tracing medullary cells from the neural tube into the ventral roots of the spinal nerves. According to his observations these cells, with similar cells which wander out from the spinal ganglia, migrate peripherally along the spinal nerves. At the origin of the communicating rami, cells deviate from the courses of the spinal nerves and give rise to the sympathetic nervous system. Inasmuch as Froriep does not admit of the existence of sympathetic sensory neurones, he concludes that all the sympathetic neurones in the sympathetic trunks and the prevertebral and the peripheral sympathetic plexuses arise from cells which have their origin in the ventral half of the neural tube and migrate peripherally along the ventral roots of the spinal nerves. My observations have shown conclusively that the cardiac plexus and the sympathetic plexuses in the walls of the visceral organs do not arise from cells which migrate peripherally along the spinal nerves, but have their origin in cells which migrate from the hindbrain and the vagus ganglia along the vagi. As I have pointed out in an earlier paper, experimental evidence indicates the existence of sympathetic sensory neurones in some of these plexuses. It is probable, therefore, that the sympathetic excitatory neurones arise from cells which migrate from the neural tube along the fibers of the motor nerve-roots, while the sympathetic sensory neurones, wherever such neurones exist, arise from cells which migrate peripherally from the cerebro-spinal ganglia. According to this interpretation, the sympathetic neurones are homologous with the afferent and the efferent components of the other functional divisions of the peripheral nervous system.

Froriep further believes that the axones which constitute the fibers of the motor roots of the spinal nerves are the vehicles by means of which medullary cells are transported peripherally along the spinal nerves, and that cells are carried from the spinal nerves into the anlagen of the sympathetic trunks by the axones which constitute the motor fibers of the communicating rami. He is not convinced as to whether such peripheral transportation is

accomplished by the peripheral growth of the axones alone or whether cells may also migrate peripherally, independently of the growth of the axones. My observations do not enable me to offer any adequate explanation of the process by which the cells giving rise to the sympathetic nervous system are carried peripherally from the cerebro-spinal system. The growing nerve-fibers, doubtless, constitute an important factor in the peripheral transportation of these elements. They are not sufficient, however, to account for the entire process alone. Nor is the presence of nerve-fibers absolutely necessary to the peripheral migration of sympathetic cells. In embryos of both birds and mammals, cells may be traced from the spinal nerves into the anlagen of the sympathetic trunks before fibers are present in the communicating rami. Likewise, cells migrate ventrally from the sympathetic trunks into the anlagen of the prevertebral plexuses before postganglionic fibers appear.

Held ('09) and Marcus ('09) have recently taken exception to Froriep's views concerning the origin of the cells giving rise to the sympathetic nervous system. Held has attempted to show, for the entire vertebrate series, that the cells present in the motor nerve-roots play no part in the development of the sympathetic nervous system. He still regards the sympathetic system as an offshoot from the spinal ganglia. In the light of the present investigation, such a position is untenable. My preparations show conclusively that medullary cells migrate into the motor nerve-roots in considerable numbers. These cells migrate peripherally along the spinal nerves just as certainly as do the cells which wander down from the spinal ganglia. Inasmuch as the great majority of the cells migrating peripherally along the spinal nerves are cells of an indifferent character, there is no reason to suppose that the cells which wander down from the spinal ganglia give rise to sympathetic neurones, while those which migrate from the neural tube along the fibers of the motor nerve-roots do not.

Marcus has attempted to show that the cells which Froriep observed in the ventral roots of the spinal nerves do not wander out from the neural tube, but migrate thither from the neural

crest. In early stages of embryos of torpedo, he has observed cell-chains connecting the neural crest with cell-aggregates in the ventral nerve-roots. He concludes, therefore, that the neural crest represents the sole source of the cells giving rise to sympathetic neurones. I have found no evidence of cells migrating from the neural crest into the ventral roots of the spinal nerves in embryos of birds and mammals. Cell-chains connecting the neural crest with the cell-aggregates in the ventral nerve-roots, doubtless, do occur in embryos of the lower vertebrates. I have observed such cell-chains in embryos of Amblystoma. This does not, however, preclude the possibility of cells migrating from the neural tube directly into the ventral nerve-roots. In the same embryos in which these cell-chains were observed, I was able to trace medullary cells from the ventral part of the neural tube directly into the ventral nerve-roots.

As has already been pointed out, the cells migrating peripherally from the neural tube and the cerebro-spinal ganglia along the spinal nerves and along the vagi are the descendants of the "germinal" cells of His; viz., the "indifferent" cells and the "neuroblasts" of Schaper. They are, therefore, homologous with the cells giving rise to the neurones and the neuroglia cells in the central nervous system. Inasmuch as some of these cells give rise to the sympathetic nervous system, the latter bears a direct genetic relationship to the central nervous system, and the sympathetic neurones are homologous with the afferent and the efferent components of the other functional divisions of the nervous system. The histogenetic relationships of the sympathetic neurones were considered at some length in my paper on the development of the sympathetic nervous system in mammals. They will, therefore, not be considered further at this point.

A comparative study of the morphogenesis of the sympathetic nervous system in birds and in mammals reveals some striking points of difference which evidently have phylogenetic significance. Two pairs of sympathetic trunks arise in the course of ontogeny in birds, while in mammals a single pair of sympathetic trunks is developed. In the early stages in mammalian embryos,

the prevertebral plexuses show their maximum development in the region of the suprarenals. In the early stages in the chick, these plexuses show their maximum development in the sacral region. This character in birds is obviously correlated with the enormous development of the ganglion of Remak which has no counterpart in mammals. Minor differences also occur in the development of the vagal sympathetic plexuses. These morphogenetic differences, doubtless, indicate that the sympathetic system has departed more widely from the ancestral type in birds than in mammals.

A study of the development of the sympathetic system in birds, as well as in mammals, warrants the conclusion that the nervous system is a unit of which the sympathetic system is a part homologous with the other functional divisions. It may be looked upon as one of the later accessions to the vertebrate nervous system which has arisen in response to the conditions of the vegetative life. The morphogenetic differences which have been pointed out in the development of the sympathetic system in birds and in mammals obviously indicate specializations in certain directions, which have arisen in response to peculiar vegetative functions.

SUMMARY

1. The primary sympathetic trunks in the chick arise about the beginning of the fourth day of incubation, as a pair of cell-columns lying along the sides of the aorta and along the dorsal surfaces of the carotid arteries. The anlagen of the secondary sympathetic trunks arise about the beginning of the sixth day, as ganglionic enlargements on the median sides of the spinal nerves. These ganglionic enlargements are at first independent of each other, but become united later by longitudinal commissures. The primary sympathetic trunks reach their maximum development during the course of the sixth day, after which they decrease in size until they disappear. The observations just summarized agree essentially with the results of His, Jr.

2. The author finds, however, that the cells giving rise to the sympathetic trunks are not derived exclusively from the spinal ganglia, as His, Jr., supposes, but that they are derived, wholly or in part, from the neural tube. Medullary cells migrate from the neural tube into the ventral roots of the spinal nerves. With similar cells which wander out from the spinal ganglia, these cells migrate peripherally along the spinal nerves. At a point a little above the level of the aorta, cells deviate from the course of the spinal nerves and, migrating toward the aorta, give rise to the primary sympathetic trunks. As migration proceeds, the cells which deviate from the course of the spinal nerves no longer wander into the primary sympathetic trunks, but become aggregated at the point of origin of the communicating rami and give rise to the anlagen of the secondary sympathetic trunks.

3. The prevertebral plexuses arise as cell-aggregates lying along the ventro-lateral aspects of the aorta from the suprarenals posteriorly. They are derived directly from the primary sympathetic trunks.

4. The ganglion of Remak arises as an oval cell-column lying in the mesentery just dorsal to the rectum. It arises from cells which the author finds to migrate ventrally from the hypogastric plexus.

5. The cardiac plexus and the sympathetic plexuses in the walls of the visceral organs, which the author has designated as the "vagal sympathetic" plexuses in an earlier paper, arise from cells which migrate from the hind-brain and the vagus ganglia along the fibers of the vagi. In the posterior region of the intestine, the myenteric and the submucous plexuses probably receive some cells from the ganglion of Remak.

6. The cells which migrate from the neural tube and from the cerebro-spinal ganglia along the spinal nerves and the vagi are the descendants of the "germinal" cells of His; viz., the "indiffer-

ent" cells and the "neuroblasts" of Schaper. They are, therefore, homologous with the cells which give rise to the neurones and the neuroglia cells in the central nervous system, and the sympathetic neurones are homologous with the afferent and the efferent components of the other functional divisions of the peripheral nervous system. These observations agree with the author's observations on mammalian embryos.

7. Certain morphogenetic differences exist in the development of the sympathetic nervous system in birds and mammals, which the author interprets as indicating that the sympathetic system has departed more widely from the ancestral type in birds than in mammals. Such departure is no more than should have been expected in the specialized avian branch of the vertebrate series.

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THE ORIGIN OF THE CRANIAL GANGLIA IN AMEIURUS

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EIGHTY-EIGHT FIGURES

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INTRODUCTION

The origin and relationship of the cerebral nerves have been, since the appearance of Balfour's classical researches on the nerves of Elasmobranchs in 1876, among the most interesting and puzzling problems in vertebrate morphology. Notwith-

standing the enormous amount of labor expended on this problem, it can hardly be said that there is any general agreement among workers as to the mode of origin or fundamental relationships of the various cerebral nerves to each other.

The lack of agreement may be traced to several causes aside from the difficulty inherent in the problem. Chief among these causes must be placed the subordination of the study of the cerebral nerves to more general problems, such as the metamerism of the vertebrate head and the relation of the head to the trunk. These fundamental problems have been a source of intense interest to both morphologists and embryologists and it was only natural that the cerebral nerves should be used as a means of shedding light on them, even when the origin, composition and distribution of the nerves were not accurately known.

Owing to this method of approach, however, students of the cerebral nerves were for a long time handicapped by preconceived notions as to the relations which ought to exist between the trunks of the cerebral nerves and the various head segments. The idea of the serial homology of the cerebral nerves has been in a sense a barrier to a clear understanding of their true relationships. The chief function of the nervous system as a coördinating mechanism was lost sight of in the attempt to reduce the cerebral nerves to some fundamental type that would bring them into harmony with each other and with the spinal nerves.

Some such fundamental relationship among the cerebral nerves as that involved in the idea of serial homology probably existed in a primitive vertebrate; but the cerebral nerves of the Ichthyopsida are not most easily understood on such a basis. The fundamental needs of the organism have so modified this primitive type that it is much easier and more in accord with the facts to take some functional unit, such as a sensory system, as a basis for the analysis of the cerebral nerves.

The fundamental difficulty in the attempt to homologize the trunks of the cerebral nerves comes from the fact that they are not units but composite in nature, so that the components of which they are made up furnish the logical basis for getting at their true relationships.

The realization of this fact and its use as a working basis in the attempt to determine the relationships of the cerebral nerves is the most striking characteristic of recent work on the nervous system of the lower vertebrates. The recognition of functional units rather than serial homologies as the keynote in this work has tended to free the study of the cerebral nerves from subordination to larger problems and to call attention to the need as well as to the possibility of a more thorough knowledge of the composition of the nerves before this knowledge can be applied to such problems as the metamerism of the head or to the relation of the head to the trunk. It has also served to bridge the gap that has existed between the structural and functional conceptions of the nerves by adopting a unit that is not only structural in character but functional as well.

Such an analysis of the cerebral nerves has been possible, owing to the fact that, among other things, there are structural differences between the various components that have made it possible to follow them throughout their whole course in favorable types. The analysis has been materially strengthened of course by a clearer understanding of the various types of end organs to which the fibers are distributed peripherally as well as of the central connections in the brain and cord.

The net result of this work seems to have changed the basis for homologizing the cerebral nerves from the nerve trunks to the nerve components. These are anatomical and physiological units characterized by similarity in function, peripheral distribution and central connections; but they are not uniformly distributed throughout the various cerebral nerves even in closely related types. Nerves with the same name and the same general topographical relations may vary totally in composition. This fact makes it evident that there could be no agreement as to the homology of the cerebral nerves as long as their exact composition was unknown.

A more thorough knowledge of the components of which the cerebral nerves are made up and of the composition and position of the ganglia from which they arise opens the question as to the origin of the discrete elements of these ganglia. Are

they as distinct from each other in their mode of origin as they are in their structure and function in the adult?

Even a cursory reading of the literature on cerebral nerves shows a well defined movement away from the idea of concreteness of origin and toward that of discreteness of origin. Recently however, so far as I am aware, no attempt has been made to follow out carefully the development of the cerebral ganglia in some type in which the nerve components are known. The present paper is an attempt to fill this gap in our knowledge by tracing the various components back to their earliest recognizable stages, keeping in mind the fact that the origin of a definitive ganglionic mass of known composition and function in the adult is the end in view. The attempt to account for the fate of all embryonic cell masses, a part of which may go to form ganglia, presents a totally different problem and one with which we are unable to cope with present technical methods.

I wish to express my gratitude to Dr. C. O. Whitman for his friendly criticism and guidance during the progress of this work.

HISTORICAL SKETCH

The following brief historical account aims to give only the important advances by which our knowledge of the discreteness in origin and structure of the cranial ganglia and nerves has grown.

According to Balfour ('75), before the appearance of his work on the spinal ganglia in Elasmobranchs, it was the prevailing opinion that the ganglia were derived from the mesoblast of the vertebrae. His had in 1868 ascribed their origin to the epiblast but his work seems not to have been confirmed up to the time Balfour published. Balfour, as is well known, traced the ganglia to the cord but believed that his work diverged from that of His no less than from that of his own predecessors. Goette and Semper, writing in 1875, however, had attributed the origin of the nerves to the epiblast and Goette had extended this observation to the head. In 1882 Van Wijhe and Hoffmann, working on teleosts, confirmed Goette's work and Van Wijhe showed that the neural

crest cells fuse with the lateral ectoderm in two places. This was confirmed for amphibia in 1886 by Misses Johnson and Sheldon, who like Van Wijhe found that the dorsal fusion is connected with the lateral line.

In 1885 Froriep described in mammals, what he designated as branchial sense organs on the facialis, glossopharyngeus and vagus nerves. The epidermal thickenings which he figures and designates as branchial sense organs are undoubtedly epibranchial placodes since they lie immediately over the gill slits and there are no lateral line nerves in the mammals. Froriep, however, thinks that the resemblance of these anlagen to those of the lateral line anlagen places them in that group of nerves. He further finds that the evidence for the addition of cells from the placode to the ganglia in these nerves is slight, since only in the earlier stages are the boundaries between the two structures indistinct.

Beard ('85, '87, '88) described primitive branchial sense organs in elasmobranchs, teleosts, amphibia, reptiles and birds and also stated that the epidermal thickening contributed cells to the ganglia derived from the neural crest. Beard rejected the term lateral line organ because the lateral lines originate in the head and substituted therefor the term branchial sense organ, and he seems always to have had in mind the lateral line organs of the head when he speaks of branchial sense organs; at least he confuses the dorso-lateral and the ventro-lateral placodes and, as von Kupffer ('91) intimates, it is impossible to tell which he is discussing at times. He figures and mentions, however, two points of contact of the neural crest ganglia with the skin.

Beard ('88, p. 882) refers to the fusion of the neural crest with the epiblast at the level of the notochord, and just above the gill cleft in the same paragraph, apparently not distinguishing between them, although in the same paper he criticizes Onodi for not having seen the second fusion of the ganglion anlage with the epiblast. It seems a safe conclusion from Beard's papers that he took all fusions of the neural crest to be concerned in the formation of the lateral nerves.

In discussing the origin of the neural crest portion of the ganglia Beard ('88) insists that the origin of the spinal ganglia from the

Zwischenstrang as described by His is not correct but that in all cases he finds the ganglion arising from a mass of cells derived from the epiblast at the point of the entering angle between epiblast and cord and that the Zwischenstrang takes no part in its formation and is present after the ganglion has detached itself from the epiblast and that the ganglion anlage can always be detected before the closing in of the medullary plates. Beard failed, however, as his predecessors had failed, to distinguish between the dorso-lateral placodes and the early stages of the adult lateral line organs. Under the term branchial sense organs he may have been describing either lateral line organs in the head, or dorso-lateral placodes which may give rise to ganglion cells. Von Kupffer, working on Petromyzon, in 1891 made a sharp distinction on the one hand between dorso-lateral placodes or those placodes concerned in the origin of the lateral line and lying at the level of the notochord, and on the other hand the epibranchial placodes which arise just over the gill slits. He also states that the epibranchial ganglia are concerned in the origin of the branchial nerves, so that one may infer from his work that there were separate origins for what we now designate as lateralis and visceral sensory nerves.

Miss Platt ('95) showed that in Necturus the lateral ectoderm gives rise not only to the lateral line ganglia and nerves which appear in three primitive longitudinal ridges, but also to mesectoderm, cells proliferated from the lateral ectoderm and from the neural crest and assuming the position and characteristics of mesoderm. Miss Platt seems first to have made a distinction between the earliest thickening of the lateral epidermis, *i.e.*, the dorso-lateral and epibranchial placodes, and the early stages of the lateral line organs. These seem to have been confused by previous workers. She says (p. 500) that "the large dorso-lateral and epibranchial ganglia are formed from cells that split off *en masse* leaving the ectoderm external to them for the time thin. A sensory ridge may appear later in the exact place where the ganglion arose, as happens in the supra-orbital line, or sense organs may form at either side of the ganglionic anlage, as in the vagus region." She calls attention (p. 497) to the fact that the growing point of

the dorso-lateral placode may simulate very closely a lateral line organ (fig. 5), but that it does not give rise directly to these organs since this growing point is found in segments where later there are no lateral line organs.

Miss Platt's work marks in a way the culmination of the work begun by Balfour in 1876. A more thorough analysis of the cerebral nerves of the adult was needed before the full significance of the dorso-lateral and epibranchial placodes could be determined. While she seems to place the dorso-lateral and epibranchial placodes in the same category, the determination that not all cells proliferated from the neural crest and epidermis go to form ganglia and that the dorso-lateral placodes do not in some cases give rise directly to sense organs, but that these appear later, mark distinct advances in our knowledge of the origin of structures derived from the epidermis. Much confusion has arisen apparently from ignorance of these facts.

Locating a specific ganglion of known composition in the adult and then tracing it to its origin would have prevented errors that have arisen unavoidably from pursuing the opposite course and finding a segmental ganglion and nerve for every area in which cells are being proliferated from the neural crest and ectoderm. As a general conclusion from her work, she thinks that the root of a sensory nerve is no index to the segmental value of that nerve, the position of the nerve root being in a great measure the expression of the coördinate relations which the central nervous system serves. Miss Platt emphasizes another point of the greatest importance; it is in connection with the distinction between definitive ganglion cells and nerve fibres on the one hand, and the anlagen from which these come on the other. Just as not all cells derived from the ectoderm go to form ganglia and nerves, so not all cells grouped about ganglia and the growing points of nerves are concerned directly in the nervous functions of the nerves and ganglia.

The appearance of Strong's paper in 1895, and more particularly of Herrick's in 1899, mark as it appears to the writer, a turning point in the study of the cerebral nerves. Evidently no homology of the cerebral nerves can be established without an exact knowl-

edge of the central ending, ganglionic relations, and peripheral distribution of the various components of these nerves. Confusing acustico-lateralis ganglia with communis or visceral ganglia or failing to distinguish between perfectly discrete regions in the brain leads only to confusion. The fact that in many types a difference in size of fibres, in addition to the characteristics mentioned above is present, has made possible the determination of the exact composition of the trunks of the cranial nerves and has made unnecessary the almost interminable and confusing discussions of their homologies. The determination by Herrick ('99) that there are in the Vth, VIIth, VIIIth, IXth, and Xth nerves of Ichthyopsida three, and apparently only three, chief groups of sensory components and the exact definition and description of these components and their ganglia marks in a general way the advance in our knowledge of cranial nerves since Strong's paper appeared.

The three components mentioned above are, first, the general cutaneous, characterized by ending in the brain in the spinal fifth tract, by having ganglia (in Menidia) in the Vth and Xth nerves situated intracranially, by having medium sized fibres and by being distributed peripherally to the skin as free nerve endings. Second, the acustico-lateralis, or special cutaneous, characterized by ending in the brain in the tuberculum acusticum, by having ganglia in the VIIth, VIIIth, and Xth nerves, by having large fibres and by being distributed to the ear and lateral line organs only. Third, the communis or visceral sensory system characterized by ending in the brain in the nucleus of the fasciculus solitarius or its equivalent in the vagal, glossopharyngeal, and facial lobes, by having ganglia in the VIIth, IXth and Xth nerves, by having small sized fibres and by ending peripherally in taste buds wherever found, and general mucous surfaces. While this paradigm holds for teleosts generally, the irregularity with which some of these components are distributed in the various cerebral trunks makes it clear why there was so much confusion and disagreement in the earlier attempts to determine the homologies of these trunks.

A comparison of Koltzoff's excellent paper ('02) on the embryology of Petromyzon with the work of Johnston ('05*b*) on the nerve components of the same type will serve to introduce the point of view from which the author has worked the early stages of the ganglia in Ameiurus. Koltzoff does not list a single nerve component paper in his bibliography and was in fact working primarily on the segmentation of the head, and only incidentally on the origin of the ganglia. The results of these two papers are summarized in the following diagram:

TABLE I

Summarizing the results of Koltzoff's work on the development of the ganglia in Petromyzon, and of Johnston's on the adult structure of the same form

		PROF. OR TRIG. I. V	TRIG. OR TRIG. II. V	VII	VIII	IX	X
Koltzoff (Derivative)	Neu. Crest	Neu. crest	Neu. crest	Neu. crest	Neu. crest	Neu. crest	Neu. crest Segmental
	D. L. Plac.	D.L. Plac.	D.L. Plac.	D.L. Plac.	D.L. Plac.	D.L. Plac.	D.L. Plac.
	Ep. Plac.			Ep. Plac.		Ep. Plac.	Ep. Plac. Segmental
Johnston (Component)	General Cutaneous	Prof. N. and Gan.	Trig. N. and Gan.	Gen. Cut. VII		Gen. Cut. IX	Gen. Cut. X. Segmental
	Acustico-Lateralis	Lateralis Prof.	Lateralis Trig.	Lateralis VII	Auditory	Lateralis IX	Lat. X. Not Segmental
	Communis or Visceral			Com. VII.		Com. IX.	Com. X. Segmental

It will be seen at a glance that every nerve into whose ganglion cells derived from the epibranchial placode enter contains in the adult visceral fibres; every nerve to whose ganglion the dorso-

lateral placodes contribute cells contains acustico-lateralis fibres in the adult; and, finally, every nerve, except the eighth, to whose ganglion the neural crest contributes cells contains in the adult general cutaneous fibres.

If it were not for the single exception in the case of the VIIIth nerve, a very natural conclusion from this comparison would be that the epibranchial placodes gave rise to visceral ganglia, the dorso-lateral placodes to acustico-lateralis ganglia and the neural crest to general cutaneous ganglia. The error in this conclusion arises from the assumption that the epibranchial and dorso-lateral placodes give rise to all of the ganglia into which they enter. This may occur in some cases but certainly does not in all. There is a very general agreement among workers on the origin of the cerebral ganglia that the neural crest cells fuse with cells derived from either the dorso-lateral placodes or the epibranchial placodes or both, so that the assumption that these placodes give rise to all of the ganglia into which they enter is not only unwarranted but contrary to evidence presented in a large number of cases. If neural crest cells enter into the auditory ganglion of Petromyzon, which is the most specialized of all the acustico-lateralis ganglia, it would not be surprising if they should enter into the less specialized ganglia, such as those of the VIIth nerve. An examination of the literature on the composition of the auditory ganglion shows a great deal of diversity of opinion as to the presence of the neural crest in the region of the auditory vesicle. Johnston ('06) states that it is absent and Rabl ('92) and Hoffmann ('94) both take the same position. On the other hand, Beard ('88), Van Wijhe ('82), von Kupffer ('94), Platt ('95) and Neal ('97) hold that it is present. There seems little doubt that it is present in some types in the earlier stages at least. It does not follow from this fact that neural crest cells enter into the auditory ganglion. Dohrn ('90) holds that the neural crest is at first continuous between the acustico-facialis and the glossopharyngeus, but later disappears, that it is sometimes present on both sides and sometimes only on one side and that the masses of cells which he sometimes finds are to be interpreted as remnants of the former connections of preauditory and postauditory neural crest. The fact that there are general

cutaneous fibres in the VIIth in Petromyzon, that the crest is at first continuous but apparently not so later, and that when the VIIth and IXth contain general cutaneous fibres they also contain neural crest cells seem to the writer to minimize the discrepancy between Kolzoff's and Johnston's work, since these cells may enter into the VIIth and IXth in Petromyzon. The matter is complicated by the fact that neural crest cells of the VIIth lie between the anterior portion of the auditory vesicle and the neural tube and come into contact with the vesicle at or near the place where the cells of the auditory ganglion are proliferated from the vesicle so that the relations are confused and it is difficult to be certain of the exact conditions. Leaving out of consideration the VIIIth ganglion, we can infer that the general cutaneous ganglia come exclusively from the neural crest, while the acustico-lateralis and visceral ganglia come in part from the dorso-lateral and epibranchial placodes respectively but may contain neural crest cells in some cases.

Without anticipating the conclusions drawn from a study of Ameiurus, it may be well to call attention to two facts: first, the acustico-lateralis system of nerves and ganglia is usually treated as a special cutaneous system on account of the fact that the tuberculum acusticum which is the acustico-lateralis center in the medulla oblongata is a specialized portion of the general cutaneous center of the spinal cord extending into the medulla oblongata. The close relationship of the general cutaneous and acustico-lateralis systems is thus based on good anatomical evidence. Second, the communis or visceral system is really double in character; it consists of a general visceral portion which supplies general mucous surfaces, and it also contains a special visceral or gustatory portion whose fibres end peripherally in taste buds, so that the presence of neural crest cells in the acustico-lateralis and visceral ganglia might be explained in the first case on the basis of the close relationship between the general cutaneous and the acustico-lateralis systems of nerves and ganglia and in the second case on the basis of the double composition of the communis or visceral system.

The determination of the relation of neural crest cells to those

cells derived from the dorso-lateral and epibranchial placodes was one of the chief objects in taking up the study of Ameiurus. Unexpected difficulties were met, owing to the fact that Ameiurus has no well defined neural crest in the head such as is found in other types, so that my conclusions are based on the assumption that there are in Ameiurus cells which correspond to the neural crest of other types. Aside from this peculiarity, it has proven to be an exceptionally favorable type. The epibranchial placodes are large and easily followed and the epibranchial ganglion of the IXth nerve seems to be a pure placodal ganglion, a fact which enables us to come to a definite conclusion in regard to the relation of the neural crest cells to those derived from the placode. It seems to be necessary to determine this point definitely before we can come to any satisfactory conclusion as to the relation of the spinal and cerebral ganglia to each other. The acustico-lateralis and gustatory nerves are peculiar to the head but the general visceral and the general cutaneous are not, being found in the trunk. The problem of the relation of spinal and cerebral nerves becomes largely a question as to how the general visceral and general cutaneous nerves are related in origin to the special visceral and special cutaneous nerves in the head. Aside from the fact that it was necessary to choose some type in which the nerve components are known, the chief reason for taking Ameiurus lies in the enormous size of the gustatory system of sense organs and nerves. The taste buds are distributed over practically the whole body surface as well as in the mouth, pharynx and œsophagus, and gustatory fibres are found in ten out of twelve of the chief nerves arising from the trigemino-facial ganglia. A type with such an enormously hypertrophied gustatory system seemed likely to furnish a good basis for determining the exact mode of origin of the special visceral ganglia and nerves.

A summary of the cranial ganglia of Ameiurus as given by Herrick ('01) is shown in the following diagram:

TABLE II

Showing the components of the Vth to Xth ganglia in Ameiurus; compiled from Herrick's ('01) description. The presence of any component is indicated by "pres," or by the name of the ganglion. The jugular and lateralis Xth are placed over the third and fourth epibranchial ganglia of the Xth not to indicate their segmental position but because this is their relative position.

	V	VII	VIII	IX	X	X	X	X
Gen. Cut....	Gass.	.....	.....	.....	.....	.....	.....	Jugular
Acus. Lat...	.....	Lat. VII.	Pres.	Pres.	.....	.....	.....	Pres.
Communis ..	.....	Pres.	.....	Pres.	Pres.	Pres.	Pres.	Pres.

This table is slightly different from Herrick's description. There is a lateralis ganglion in the region of the IXth nerve which he seems to attribute ('01, p. 208) morphologically to the Xth, although it is associated with the root of the IXth. I find it to be entirely distinct in origin from the Xth nerve and have placed it in the diagram with the IXth. The general cutaneous ganglia are found in the Vth and Xth nerves. The acustico-lateralis ganglia are found in the VIIth, where there are two divisions, the dorso-lateral and the ventro-mesial; and in the VIIIth, IXth, and Xth. The visceral ganglia are found in the VIIth, IXth and in the four branchial ganglia of the Xth. The jugular or general cutaneous Xth and the lateralis Xth are placed in the diagram over the last epibranchial ganglion of the Xth, not to indicate their segmental position but because they form the posterior portion of the vagus ganglion. Attention will be called later in the body of the paper to the fact that this table makes no distinction between the general and special visceral ganglia, both being catalogued as the visceral sensory or communis system, although Herrick distinguishes the two groups functionally and further distinguishes the two types of fibres structurally.

MATERIAL AND METHOD

In a type which develops so rapidly as Ameiurus, there being only five days between fertilization and hatching, the age seems to be a better way to designate a series than the body length. Series must be taken at close intervals and, while individuals of a given age may not vary by an appreciable amount in length, there are frequently found in the same series quite perceptible variations in the degree of differentiation of the ganglia and sense organs, so that while the age is not an absolutely accurate method of designating a series since the growth varies with temperature, I have used this on account of the difficulty of separating embryos by their length.

As to method, I have followed consistently the plan of locating definite ganglia in older series after they were well defined and tracing these back to the earliest recognizable stages. This plan seems to be absolutely necessary, since only in a few cases do the definitive ganglia use all of the material from which they are formed and in some cases, particularly the general cutaneous ganglia, only a very small portion of the mass from which the ganglion is formed finally enters into the composition of the ganglion. Some confusion seems to have arisen, especially in the earlier work on nerves and ganglia, from taking it for granted that all of the material from which a ganglion forms enters into a ganglion.

The material on which this work was done is in part, the same as that used in a former paper by the author (Landacre, '07) and consists of series of Ameiurus melas of which the absolute age is known since the process of oviposition was observed; and in part, particularly the early stages, the work was done on a large number of graded series of Ameiurus nebulosus of which the relative age was known but the absolute age was not known. The series of Ameiurus melas are indicated by their ages. Of the nebulosus material nine stages were used of which the last two series, VIII and IX, are quite similar to each other and correspond closely to the forty-nine hour series of A. melas. Series I seems to be about twenty-four hours old compared with a similar stage of A. melas. The remaining series II to VII inclusive are all younger

than the youngest of the A. melas series, which was forty-nine hours old. In the following brief characterization of the stages the optic cup, the optic lens, and the auditory vesicle are used chiefly as a means of separating them in addition to the more minute differences to which attention is called in the body of the paper.

Stage I. An early stage (24 hours?) of A. nebulosus in which the blastoderm is nearly flat, the neural keel being elevated slightly above the yolk at the anterior end only. The blastoderm has not extended sufficiently far posteriorly and ventrally to be cut on the ventral side of the yolk sac at the posterior end of the embryo. Fig. 1 was drawn from this stage.

Stage II. Optic vesicle present, but solid. Nuclei of cells in optic vesicle uniformly distributed with no indication of the peripheral arrangement of the nuclei which precedes the formation of a cavity. Nuclei of auditory vesicle irregularly arranged with no indication of a cavity. The auditory vesicle can be located only by comparison with an older series. Lateral mass not broken down into mesectoderm at any point. Figs. 2 and 3 are drawn from this stage.

Stage III. Optic vesicle solid but with nuclei of cells uniformly arranged around the periphery of the vesicle preparatory to the formation of the optic cup. Auditory vesicle with its cells elongated and the nuclei arranged around the periphery. The lateral walls of the cup not in contact, the future cavity of the cup filled with spherical cells. The solid lateral mass of the preceding stage changed into a loose mass of tissue in the region of the Gasserian ganglion, particularly just anterior and posterior to this point. The pre- and postauditory placodes are present. Figs. 4, 5, 6, 7, 8, 9 were drawn from this stage.

Stage IV. Optic vesicle with slight cavity. Epidermis not thickened preparatory to the formation of a lens. Auditory vesicle with a slight cavity and the lateral walls of the vesicle in contact. Preauditory placode just detached fom the auditory vesicle. Postauditory placode still in contact with the auditory vesicle. Gasserian and geniculate ganglia not sufficiently well defined to determine approximately their boundaries. Hyoid gill pocket

not yet in contact with the epidermis. Figs. 14, 15, 16, 44, 45, 46 were drawn from this stage.

Stage V. Optic vesicle and optic stalk completely open. Lens indicated by slight thickening of the epidermis. Auditory vesicle with well defined cavity and the proliferation of cells from the vesicle to form the ganglion beginning. Gasserian and geniculate ganglia distinguishable from the loose lateral mass cells surrounding them, although their boundaries cannot be definitely determined. Hyoid gill pocket in contact with the epidermis. First stage in the proliferation of cells from the auditory vesicle to form the lateralis IX ganglion. Hyoid gill pocket in contact with the epidermis. Figs. 10, 11, 13, 17, 18 and 63 were drawn from this stage.

Stage VI. Lens thickening well defined but changing gradually at the borders into epidermis, *i.e.*, not sufficiently thick to have its borders well defined. First epibranchial placode appears. Hyoid gill pocket still in contact with the epidermis. Figs. 19, 20, 21, 22, 23, 24 were drawn from this stage.

Stage VII. Lens constricted sharply at its borders preparatory to its detachment from the epidermis. Nuclei of cells in lens not yet located in the periphery of the cells. First epibranchial placode is proliferating cells into the mesoderm to form the first epibranchial ganglion but the ganglion is still in contact with the epidermis. The jugular ganglion of the X can be located. The lateralis X ganglion is in process of being proliferated from the postauditory placode. Hyoid gill pocket still in contact with the epidermis. Figs. 12, 25, 26, 27, 28, 29, 30, 40, 41, 42, 43, 47, 48, 49, 50, 51 were drawn from this series.

Stages VIII—IX correspond closely to a forty-nine hour embryo of Ameiurus melas being possibly slightly younger. There seems to be no well defined distinctions between stages VIII–IX or between these and A. melas forty-nine hours. The three figures drawn from these two series could have been taken from A. melas. Fig. 61 was drawn from Stage VIII and figs. 52, 62 from Stage IX.

All sections were cut 7μ thick and it will be convenient to express the spatial relations of structures in sections.

THE DIFFERENTIATION OF THE NEURAL PLATE

Ameiurus presents a rather striking difference from the descriptions usually given of the formation of the neural cord in its relation to the origin of the neural crest and dorso-lateral placodes.

Balfour ('75) described the neural crest as growing out of the neural cord but seems not to have worked stages sufficiently early to determine its exact mode of origin. Marshall ('77) gives substantially the same description of its origin. Beard ('88), however, describes it as arising in elasmobranches, teleosts, amphibians, reptiles and birds as a thickening of the epidermis, lying lateral to the neural plate and always distinguishable from that structure before the neural tube is formed. There is substantial agreement among all the earlier descriptions of the cerebral ganglia in attributing to the neural crest a definite structure related more or less closely to the dorsal portion of the neural plate as it folds off to form the neural cord (Harrison '01, text-figs. 1–5). Few authors, so far as I am aware, have found any close relation between the neural crest and dorso-lateral placodes in their earliest stages. The neural crest ganglion is almost always described as growing down ventrally from its point of origin and coming into contact with the epidermis at the point of origin of the dorso-lateral placode on a level with the notochord. Wilson and Mattocks ('97, p. 659) do, however, describe the dorso-lateral placode of the salmon as arising not by a thickening of the epidermis but by the thinning out of the neural shield which leaves the placode isolated, lying in a lateral position. In the salmon the placode is at first located in the lateral portion of the neural shield just as in Ameiurus.

The early stages of Ameiurus differ from the usual descriptions in that the neural crest and dorso-lateral placodes are not differentiated from each other at first but appear as a large lateral thickening lying on either side of the neural plate. This I have designated as the *lateral mass* (fig. *A*.) It doubtless contains regions comparable to the neural crest and certainly to the dorso-lateral placodes of other authors; but since these are not recognizable in the early stages and in fact the neural crest is never recognizable

as a structure distinct from the lateral mass, and since the lateral mass contains much material that does not go to form ganglia before undergoing differentiation and has a definite structure and position of its own, it seems better to characterize it as indicated above.

The lateral mass in an early stage in which the neural plate is still nearly flat has the appearance indicated in fig. 1. In Stage

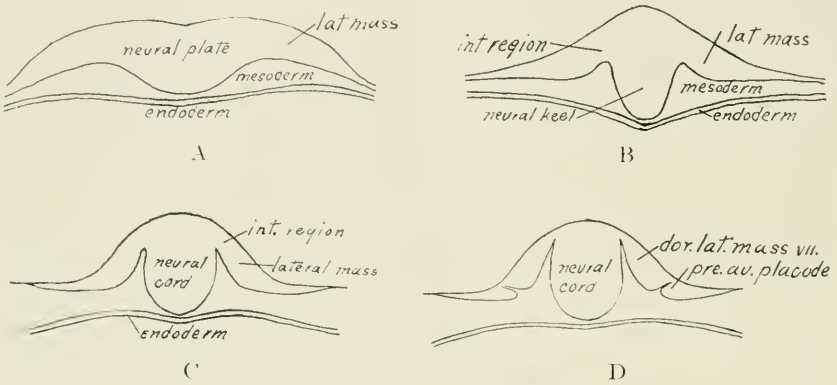


Fig. A. A camera tracing of the blastoderm corresponding to fig. 1, showing the broad, flat neural plate.

Fig. B. A camera tracing corresponding to fig. 3. showing the position of the lateral mass and of the intermediate region.

Fig. C. A camera tracing four sections anterior to fig. 9, showing the relation of the lateral mass to the neural cord.

Fig. D. A camera tracing corresponding to fig. 9, showing the relation of the preauditory placode to the dorso-lateral mass. Posterior to this point of a few sections, the auditory vesicle occupies the position of the preauditory placode and dorso-lateral mass.

II (fig. *B*), it is separated from the neural keel by a thinner region which I shall designate as the intermediate region to distinguish it from the lateral mass. This thickening extends throughout the whole length of the head and into the cord region. The lateral mass does not become incorporated into the neural cord which forms from the thick central mass, or neural keel, lying between the intermediate regions on either side.

As the neural keel deepens and assumes the form of a cord (fig. *C*), and as the blastoderm rises on the yolk and assumes a rounded form, the lateral cell masses are brought gradually into a lateral position, still retaining their connection with the dorsal half of the cord by an intermediate slightly constricted area.

In an embryo in which the optic vesicle has reached a stage in which the future optic cup is slightly larger than the stalk (Stage II), this lateral thickening begins some five or six sections posterior to the stalk and extends from this point back beyond the region in which the Xth ganglion is later formed, more than one hundred sections. Posterior to this point where the keel is forming in the region of the spinal cord it gradually becomes reduced in size as the keel becomes shallower. Throughout this whole region the lateral cell mass has a structure quite uniform at first, varying only in shape, being somewhat thicker and more closely applied to the sides of the brain in the anterior region (fig. 2), and somewhat thinner and more dorsally attached to the cord in the posterior region, particularly posterior to the position in which the auditory vesicle develops.

Six sections posterior to the optic vesicle (fig. 2), the lateral mass is applied to the dorsal half of the cord and is homogeneous in structure. This section lies in the region anterior to that in which the Gasserian ganglion later appears. In the region in which the Gasserian ganglion forms and posterior to it (fig. 3), the lateral mass is broader and thinner and the attachment to the neural cord is less extensive. This condition of the lateral mass persists throughout the region in which the Gasserian ganglion forms and back of this until we come to the region just anterior (fig. 4) and just posterior (fig. 5) to the auditory vesicle, where, in a slightly older embryo (Stage III), there is soon noticeable a slight differentiation of the lateral mass into a thicker dorsal portion, the dorso-lateral mass (*D. L. M.*, figs. 4 and 5), connected with the cord by the intermediate region, and a ventral mass (*Pre. Pl.*, fig. 4, *Post. Pl.*, 5) slightly separated from this dorsal mass on its mesial border by a constriction. This ventrally differentiated mass, or dorso-lateral placode (pre. au. placode, fig. *D*) is present throughout the whole auditory region (fig. 6),

and extends somewhat anterior and posterior to the auditory region, where it becomes merged completely with the dorso-lateral mass to form the lateral mass. Posterior to the auditory vesicle, in the region of the IXth nerve, it presents the appearance shown in fig. 5. This ventrally differentiated mass shown in figs. 4 (*Pre. Pl.*), 5 (*Post. Pl.*) and 6 (*Au. Ves.*) develops later into the auditory vesicle and the pre- and postauditory placodes (dorso-lateral placodes).

The fate of the lateral mass, as a whole, varies in different regions of the head. In the regions between the optic stalk and the Gasserian ganglion it becomes converted entirely into mesectoderm. Fig. 7 is taken from an embryo slightly older (Stage III) than that from which figs. 2 and 3 were taken, and is identical in position with fig. 2, with which it should be compared. The lateral mass is here free from the cord on its mesial border nearly to the dorsal surface of the cord; while on its lateral border it is free from the epidermis up to about the same level. The ventral two-thirds of the mass is converted into a rather loose mass of mesectoderm in which the cell boundaries are indefinite and in which there are numerous intercellular spaces.

The later history of this mass shows that it is converted completely into a very loose mass of mesectoderm with large intercellular spaces and with faint cell boundaries, but with well defined nuclei. I have detected during this change of the lateral mass into mesectoderm no mitotic figures in any of my sections. Posterior to the region in which the Gasserian ganglion forms and between that ganglion and the lateralis VIIth the lateral mass is converted chiefly into mesectoderm, except its ventral border which represents the forward extension of the primordium of the auditory vesicle, or the preauditory placode. Fig. 8 from the same embryo is taken through the region in which the Gasserian ganglion forms. The lateral mass is here detached from the cord mesially, except at its dorsal border, but it is attached to the epidermis throughout its whole length. The dorsal and particularly the dorso-mesial portion of the solid lateral mass is beginning to be converted into a looser cell mass. Fig. 9 is taken just anterior to the auditory vesicle in the position in which the lateralis VIIth ganglion will

appear. The dorsal portion of the lateral mass which is still quite solid and has definite cell walls is later converted into the lateralis VIIth ganglion and possibly in part into the anterior portion of the auditory ganglion. The ventral portion of the lateral mass (fig. 9, *Pre. Pl.*) is slightly differentiated from the dorsal and represents the preauditory placode. As one reads back in the same series this preauditory placode becomes larger (fig. 4) and the dorsal portion of the lateral cell mass smaller until the auditory vesicle is reached (fig. 6.) The change in size of the preauditory placode is almost imperceptible. It extends farther and farther dorsally until the condition of fig. 4 is reached. The auditory vesicle here has not yet incorporated all the lateral cell mass; at least all the cells of the lateral mass have not yet assumed the radial form with distally arranged nuclei which is so characteristic of the auditory vesicle.

The future lateralis VIIth ganglion is at this time quite large in front where it lies dorsal to the preauditory placode, while posteriorly it becomes smaller and assumes a dorsal and mesial position with reference to the placode. It does not extend posteriorly beyond the anterior end of the vesicle. In the region of the vesicle the whole lateral cell mass is converted into the auditory vesicle.

From this lateral cell mass are differentiated directly, first the mesectoderm lying immediately anterior and posterior to the Gasserian ganglion and posterior to the auditory vesicle. Secondly it gives rise to the Gasserian ganglion and posterior to the ear gives rise to the jugular ganglion. It also gives rise to a large part of the geniculate ganglion, excepting of course the placodal portions, and to the greater portion of the visceral ganglion of the Xth; all of it, in fact, except those portions derived from the third, fourth, fifth and sixth epibranchial placodes. In contrast with these structures which are derived primarily from the lateral mass, we have the VIIIth ganglion and the lateralis IXth which come largely, if not exclusively, from the auditory vesicle, and the lateralis Xth which comes exclusively from the postauditory placode. These may be considered as coming secondarily from the lateral mass, the auditory vesicle and postauditory placode representing the primary derivatives from this structure.

THE DIFFERENTIATION OF THE GASSERIAN GANGLION

As mentioned above, the lateral mass has at first a perfectly homogeneous structure from the region just behind the optic stalk throughout the whole length of the head.

At the time when the auditory vesicle first becomes recognizable the lateral mass anterior to the vesicle is characterized by the presence of intercellular spaces and the partial detachment of the mass from the neural canal and epidermis (fig. 7). At this stage the Gasserian ganglion is not recognizable. It becomes recognizable first by the thinning out of the mesectoderm anterior and posterior to it rather than by any change in the region of the ganglion. While the whole region from the optic stalk to the auditory vesicle is in a condition much like that represented in fig. 7, the Gasserian ganglion cannot be located.

A little later, however, when the mesectoderm has become somewhat thinner the Gasserian ganglion can be located as a somewhat condensed mass of cells extending from the region of the thirty-third section back of the optic stalk to a point just posterior to the endodermic evagination of the hyoid gill-cleft. When first recognizable it occupies an area of about 12 to 15 sections and does not lie exactly parallel with the long axis of the body, its anterior end being somewhat more ventrally situated than its posterior, which lies above the level of the middle of the neural canal. There is nothing resembling a root at this stage. The whole of the posterior end of the ganglion is somewhat nearer the cord than the epidermis, but neither the root nor the trunk of the nerves from this ganglion appear for some hours. Anteriorly, as well as posteriorly, the ganglionic mass passes gradually into mesectoderm. The anterior portion of the Gasserian ganglion comes quite close to the ectoderm but there is no epibranchial placode formed in connection with this ganglion. The contact where it occurs is to be interpreted as a failure of the lateral mass to separate completely from the epidermis. The same is true of the lateral portions of the ganglionic mass. Fig. 10 (Stage V) is taken through the posterior third of the ganglion just over the hyoid gill pocket and shows the characteristic indefinite outline of the ganglionic mass shortly after it can be first recognized.

Its general shape is, in transverse section, at this time circular except at its anterior end where it is elongated dorso-ventrally and lies quite close to the epidermis. The denser appearance of the cytoplasm, with indefinite cell boundaries, is characteristic of nearly all the early ganglionic masses in Ameiurus. The later history of this ganglion is quite easy to follow. It becomes more definite in outline with cleaner borders and is quite distinct from any other ganglionic masses and cannot be confused with them. While in the early stages the posterior end of the Gasserian ganglion overlies that portion of the lateral mass from which the geniculate ganglion is derived, they are not in contact until about the 86th hour in A. melas (fig. 83). For a long time after the eighty-sixth hour, while the two ganglia are in contact, their outlines are quite distinct. Preceding this stage they are not even in contact with each other. The fibrillated root of the Gasserian ganglion appears at its posterior end where the ganglionic mass lies nearest the brain as described above, and can first be detected in an embryo of 75 hours (A. melas).

The development of this ganglion from the lateral mass is a very definite feature of the embryology of Ameiurus. There can be no doubt that it does not use all of the lateral mass in its formation and that it does not come from a neural crest as that term is generally used, although some portion of the lateral mass may be homologous with the neural crest of other types. The origin of structures which in other types come from the neural crest, and of dorso-lateral placodes from a common primordium in Ameiurus seems to be due to the failure of these structures to differentiate in the early stages of the lateral mass, and if it were not for the fact that so much of the lateral mass becomes converted into mesectoderm one might speak of the neural crest region of the lateral mass: but in no region is all of the lateral mass converted into a ganglion and since the specific structures derived from the lateral mass are ganglia, mesectoderm, auditory vesicle and placodes, it would only introduce confusion into the description to refer to specific portions of the lateral mass as neural crest, although in other types some of these structures are known to come from the neural crest.

THE ORIGIN OF THE LATERALIS VII GANGLIA

The origin of the lateralis VIIth ganglion resembles closely the origin of the Gasserian with the exception that the lateral mass (figs. 4 and 9, Stage III) giving rise to the lateralis VIIth never in my series breaks down so completely into a loose mass of cells as does the Gasserian. There is no break in continuity between the lateral mass cells and the ganglion. Posterior to the hyoid gill cleft, for some distance, the greater portion of the lateral mass breaks down into mesectoderm, between Stages III and V, and it is difficult to assign any definite boundary to the anterior end of the lateralis VIIth ganglion at first. Its anterior end, as in the case of the Gasserian, is situated more ventrally than the posterior end and comes into contact with the mesoderm of the mandibular arch anterior to the middle region of the arch. The anterior portion of this lateral mass later forms part of the geniculate ganglion of the VIIth nerve, but at this time the anterior limit cannot be outlined, not, in fact, until the placodal portion of the VIIth is detached from the epidermis, which is some hours later (A. nebulosus, Stage VIII). The whole ganglionic mass has an upward and backward trend, finally coming into contact with the auditory vesicle on its anterior, mesial and ventral walls. The whole ganglionic mass of the lateralis VIIth is quite homogeneous in structure, and shows no evidence of separating into the two ganglionic masses, the dorso-lateral and ventro-mesial, of which it is later composed. Its anterior boundary when it can be determined is not overlapped by the Gasserian ganglion but is overlapped by its root. Its posterior end, just where it comes into contact with the vesicle, is more closely applied to the neural tube than to the epidermis. The fibrillated root appears later at the posterior end, where it is in contact with the middle region of the cord.

Fig. 11, from the same embryo as that from which fig. 10 was taken, lies four sections anterior to the auditory vesicle. The lateralis VIIth ganglion shows the same irregular boundaries that characterized the Gasserian ganglion and is surrounded on all sides, except that next to the epidermis, by loose mesectoderm

in which cell walls are indistinguishable. The cell boundaries are still recognizable in the ganglion, however, showing its continuity with the lateral cell mass (see fig. 9). During all the earlier stages of this ganglionic mass it is impossible to locate definitely either its anterior or posterior limits. Its posterior end is in contact with the anterior end of the auditory vesicle at first and for some time also with the auditory ganglion, while its anterior end as mentioned above is in contact, if not continuous, with the posterior end of the geniculate ganglion. It is not until about the eighty-sixth hour that the boundaries of the two portions of the lateralis VIIth ganglion, the dorso-lateral and the ventro-mesial, can be determined; these boundaries are shown in figs. 34 to 37 and in fig. 83.

While these two divisions of the ganglion in the early stages are fused and their boundaries are difficult to determine, there is no difficulty in following the history of the lateral mass up to the time that the definitive ganglia appear. The principal variation which I have observed is in the length of the dorso-lateral portion which is always better defined than the ventro-mesial and sometimes extends well forward over the geniculate; in other cases it is quite short. One of the principal difficulties in determing definite boundaries in the lateralis VIIth ganglion comes from the fact that the root of the geniculate extends throughout the whole length of the lateralis VIIth and enters the brain at almost the same point as the root of that ganglion.

The general appearance and form of the Gasserian and lateralis VIIth ganglia resemble closely the conditions as described by Miss Beckwith ('07) in Amia, although she did not describe stages sufficiently early to determine whether the so-called neural crest arises along with the auditory vesicle as the lateral portion of the neural plate or whether the auditory vesicle arises as a thickening of the lateral epidermis and the neural crest as a more dorsally situated derivative of the epidermis. The tendency of the masses out of which the Gasserian and geniculate ganglia later form to break down first into loose tissue seems to be present in Amia also.

The origin of the Gasserian ganglion, which is a pure general cutaneous ganglion, and of the lateralis VIIth, which is a pure acustico-lateralis ganglion, from the lateral mass in adjoining

segments receives its best explanation in the interpretation given to the acustico-lateralis system as a special cutaneous system. This system is a special cutaneous system in the sense that its center in the medulla, the tuberculum acusticum, is a specialized derivative of the dorsal horn, or general cutaneous column, of the cord (Johnston '05*b*). While the ear and the lateral line organs are unique in structure and function and the acustico-lateralis fibres can be distinguished from general cutaneous, the two systems stand in the relation indicated above on account of the relation of their central endings. This view is materially strengthened by the fact that the ganglia of both systems in the case of the Vth and lateralis VIIth have similar modes of origin.

Owing to the intimate relation of the auditory vesicle and placodes with the lateralis VIIth ganglia, structurally and in point of time, I shall leave that portion of the lateral mass anterior to the lateralis VIIth and posterior to the Gasserian ganglion, *i.e.*, the communis VIIth or geniculate ganglion to be taken up in connection with the epibranchial ganglion of the VIIth nerve. From the time that the lateralis VIIth assumes definite form until the visceral VIIth appears this remnant can be located but not sharply defined. It lies just over the mesoderm of the hyoid gill bar and is somewhat denser than the more dorsally situated mesectoderm but does not differ sufficiently from either the mesectoderm or the mesoderm to enable one to determine its exact boundries. It is less dense than the more ventrally situated mesoderm.

THE AUDITORY VESICLE AND AUDITORY GANGLION

It is not necessary to descibe the auditory vesicle in detail but I have examined it carefully to determine if there is present any portion of the lateral mass in this region in addition to that portion forming the auditory vesicle and also to determine the exact mode of origin of the auditory and lateralis IXth ganglia. I find that the whole of the lateral mass in the auditory region is converted into the vesicle and that the greater portion of the auditory ganglion comes from the anterior end of the vesicle, but that the ganglion lies in such close proximity to the lateralis VIIth that

it is not possible to determine definitely whether there are lateral mass cells in the VIIIth or not, since it does not become distinct from the lateralis VIIth for some hours. Fig. 6 is characteristic of the median portion of the auditory vesicle before a definite cavity appears, at which stage it resembles the pre- and postauditory placodes (figs. 14, 45). The ventral portion of the vesicle is at this stage (fig. 6) well defined, while the dorsal portion is not yet fully differentiated from the lateral mass. The character of the lateral mass in the anterior third of the vesicle at this stage is quite like that of fig. 5 which is taken just posterior to the auditory vesicle. At the extreme posterior end where the differentiation into dorso-mesial and ventro-lateral or placodal regions has not yet appeared it resembles the condition shown in fig. 3. The ventral and mesial portions of the vesicle (fig. 6) are characterized by having elongated radially arranged cells with their nuclei situated at the periphery. Ventro-laterally the elongated cells pass gradually into the epidermis. Dorsally, however, the elongated radially arranged cells pass into a mass of irregular cuboidal cells which on its ventral border is faintly delimited from the neural tube but dorsally becomes continuous with the tube.

The next figure (12, Stage VII), taken from a slightly older embryo, shows that this mass is practically all incorporated into the vesicle, there being a few scattered cells that possibly may be converted into mesectoderm. As to the fate of the cells connecting the vesicle with the medulla there is another possible way in which they may be disposed of, that is, they may be incorporated into the medulla. There is no way of determining whether this is done, however, and the appearance indicates that they become part of the vesicle. The important fact here is that there are no lateral mass cells left in the region of the vesicle that could be homologous with the neural crest of other authors. Fig. 12 is typical for the whole length of the vesicle at this stage.

In comparing Koltzoff's ('02) and Johnston's ('05*b*) work on Petromyzon attention was called to the fact that the only point of disagreement was in regard to the presence of a neural crest which Koltzoff finds in the auditory region, and that an examina-

tion of the literature throws little light on the question. Dohrn ('90), however, taking this matter up specifically states that the neural crest is present at first in the auditory region but that it disappears. It was hoped that Ameiurus would throw light on the question, but the fact that there is no specific neural crest in Ameiurus renders my conclusions somewhat unsatisfactory in this matter. Since, however, there is no remnant of the lateral mass left in the auditory region after the vesicle forms, we may conclude that whatever the relation of the neural crest of other types to the lateral mass of Ameiurus the position taken by Dohrn is strongly supported by the evidence from this type and I find myself in agreement with Johnston ('06) when he states that the neural crest is absent in the auditory region. This statement will not hold for the region immediately anterior to the auditory vesicle, as was shown in describing the differentiation of the preauditory lateral mass. The lateral mass there furnishes the two lateralis ganglia of the VIIth nerve.

The exact mode of origin of the VIIIth ganglion is complicated by the fact that, while it seems to come almost entirely from the anterior end of the auditory vesicle, it develops in such close proximity to the preauditory lateral mass which gives rise to the lateralis VIIth ganglia and is so closely fused with the ventromesial ganglion of these two ganglia that it is impossible to be certain of its exact composition. In the stage in which the auditory vesicle is first recognizable by the radial arrangement of its cells there is no mass of cells occupying the position of the future auditory ganglion; this, when it appears as a definitive mass, is situated as a cap of cells adhering closely to the ventral and ventromesial portion of the anterior portion of the vesicle and extending slightly beyond the anterior end. Here it comes into contact with the irregular mass of cells which differentiates later into the lateralis ganglia of the VIIth nerve. At this stage before the appearance of the auditory ganglion and up to the time the preauditory placode separates from the vesicle the anterior walls of the vesicle are well defined and there is no evidence that cells are being proliferated from it. However, shortly after the separation of the preauditory placode from the vesicle the walls of the anterior

end of the vesicle lose their characteristic regular outline, while throughout the remainder of the vesicle its walls are still characterized by their clean cut boundary and by the presence of a single row of distally located nuclei. At the anterior end, however, the ventral wall becomes indistinguishable and its nuclei become several layers deep and there is no perceptible boundary between the vesicle wall and the forming VIIIth ganglion.

Fig. 13, Stage V, taken from the same embryo as figs. 10 and 11, lies four sections back of the anterior end of the auditory vesicle. The mingling of the vesicle and ganglionic cells and the numerous mitotic figures in this location indicate without doubt that the vesicle contributes cells to the ganglion. If it were not for the fact that the vesicle is constantly in contact with the ganglionic mass derived from the lateral mass, the lateralis VIIth ganglion, and seems to grow forward into this mass on its dorsal and mesial wall, one would be inclined to think that the whole auditory ganglion came from the vesicle. The conditions here are identical with those at the posterior end of the vesicle where the lateralis IXth ganglion is formed. That ganglion is proliferated from the wall of the vesicle after the vesicle is formed and can be followed from its first appearance until the vesicle ceases to contribute cells to it. The conditions at the posterior end of the vesicle are not complicated to the same extent by the presence of any contiguous ganglionic mass and one can be much more certain that the whole ganglion comes from the vesicle. Since, however, at the anterior end of the vesicle the cells derived from the vesicle are in contact with those derived from the lateral mass and there is no definite division into an VIIIth ganglion and two lateralis VIIth ganglia for some time after this, it is impossible to say positively that the whole of the VIIIth is derived from the vesicle. This mode of derivation is indicated by the conditions, however, and is further strengthened by the positions of the lines of cleavage separating the VIIIth from the lateralis VIIth which appear later, as well as by the manner in which the VIIIth ganglion adheres to the vesicle for a long time (figs. 35 to 39 and fig. 83).

I shall defer a discussion of the various ways in which the acustico-lateralis system of ganglia arises until after the description

of the origin of the lateralis Xth. The fact that there may be lateral mass cells in the VIIIth, however, does not affect the homogeneity of this system of ganglia, since the acustico-lateralis system is based on anatomical and physiological characters. We have lateralis ganglia, as in the case of the VIIth, derived solely from the lateral mass, probably from the neural crest in other types, and on the other hand lateralis ganglia, as in the case of the lateralis Xth to be described later, derived solely from the postauditory placode which is the posterior extension of the auditory vesicle. The VIII may be intermediate between these two extremes, since it possibly derives cells from both sources. The diversity in mode of origin shown by acustico-lateralis ganglia emphasizes the fact that the ultimate basis for the establishment of this system of ganglia and nerves rests on anatomical characters and on the central connections of this system in the brain and not on embryological evidence, since some of its ganglia come directly from the lateral mass like the general cutaneous ganglia, while others arise secondarily, coming from the auditory vesicle or placodes, and show a more specialized mode of origin.

There is a great deal of variation in the extent to which these three ganglionic masses (VIIIth and two lateralis VIIth) become separated from each other at any given stage. Sometimes the dorso-lateral VIIth is free at one or both ends, while in other embryos of the same age one or both ends may be incompletely separated from the adjoining ganglia. There is also much variation in the relative lengths of the two divisions of the lateralis VIIth, the dorso-lateralis VIIth sometimes extending far foward on the lateral surface of the Gasserian. Up to the 86-hour stage which I have plotted and in which the three divisions are isolated (fig. 83) the variations strike one as representing different degrees of isolation simply, and in this stage are in such a condition as Herrick ('99) describes for the acustico-facial complex in Menidia. After the 86-hour stage the ganglia seem to be in various stages of assembling into the adult condition of Ameiurus in which they are much more closely fused than in Menidia. In fact, the 86-hour stage of Ameiurus seems to be in about the same condition as the adult ganglia of Menidia.

THE FATE OF THE PREAUDITORY PLACODE

Under the term preauditory placode I include the anterior extensions of the auditory vesicle from the point where the vesicle narrows at its future anterior boundary to the extreme anterior limit of this extension. At the time when the anterior and posterior boundaries of the vesicle can first be determined by the radial arrangement of its cells and by the size of the vesicle, the placode is represented by a slightly differentiated region in the ventral portion of the lateral mass. Fig. 4, Stage III, is taken four sections anterior to the auditory vesicle; at this stage the preauditory placode occupies the whole of the ventral portion of the lateral mass. Its shortest diameter lies in the dorso-ventral plane and the walls of its cells are quite distinct. The mesial surface of the placode is in contact with mesectoderm cells which seem to have been proliferated from its surface in this early stage. Farther forward (fig. 9, Stage III) the placode is reduced in size and its separation from the lateral mass is less distinct. Slightly anterior to this point it can no longer be detected, having merged completely with the remainder of the lateral mass.

The later history of this placode shows that for a time it becomes more definite in appearance, simulating closely a lateral line organ and on a small scale the changes in the auditory vesicle, and then later disappears entirely, the greater portion of it being converted into mesectoderm. It does not give rise to either ganglia or lateral line organs. The lateralis ganglia anterior to the vesicle can be located before the preauditory placode disappears and the preauditory lateral line organs do not appear for some hours after the disappearance of the last remnant of the placode. The first trace of lateral line organs in A. melas appears in an embryo of 75 hours. There is no trace of the preauditory placode left in an embryo of 49 hours. In A. nebulosus the last trace of the preauditory placode disappears some hours before this (Stage V), so that there is a period intervening between the disappearance of the last trace of the preauditory placode and the appearance of the first primordium of the preauditory lateral line organs of more than 26 hours..

The process of disappearance is as follows: When the preauditory placode is at its maximum size it extends from the anterior end of the auditory vesicle as far forward as the point where the hyoid gill pocket comes into contact with the ectoderm. Its posterior end is at first continuous with the auditory vesicle and its anterior end gradually thins out into ordinary epidermis. Fig. 14, Stage IV, is taken just anterior to the auditory vesicle at a time when the placode is at its maximum size. The radial arrangement of the cells of the placode and the partial formation of a cavity corresponding to the cavity of the auditory vesicle are evident. This figure should be compared with fig. 6, Stage III. In fact, in some series there is a small cavity in the placode corresponding to that of the auditory vesicle. The first change of a retrogressive nature that I can detect is illustrated in fig. 15, which is taken from the opposite side of the same embryo. Here there is an area corresponding to the thickness of one section intervening between the posterior end of the placode and the anterior end of the auditory vesicle. Here the placode seems to have broken down into mesectoderm, since we have only mesectoderm with a trace of the ventral portion of the placode left, where on the opposite side the placode and vesicle are continuous. The irregular outlines of the posterior end of the placode and of the anterior end of the vesicle also indicate that the placode has been converted into mesectoderm and that the two structures have not simply been carried apart by the growth of the embryo. It is not likely that the placode has moved forward bodily, since the anterior end remains constant in position.

In the next stage sketched from an embryo of the same age but slightly more developed, there is an area of six sections intervening between the posterior end of the placode and the anterior end of the vesicle where the placode has been converted into mesectoderm. The placode here (fig. 16, Stage IV) differs somewhat in appearance, being longer in its dorso-ventral axis and resembling less the auditory vesicle. While the nuclei are still situated in the inner extremities of the cells, the placode has the appearance of columnar epithelium. In a somewhat older series (fig. 17,

Stage V) the placode at this point has broken down almost completely into mesectoderm. The former position of the placode (*Ep*) is indicated by the slight elongation of the remaining cells and the presence of the small cavity which frequently exists on the outer surface between the elongated cells and the flattened layer of the epidermis. This section is taken nineteen sections anterior to the auditory vesicle and there is an area of 18 sections between the anterior end of the vesicle and the posterior end of the placode in which the placode has been converted into mesectoderm. The fate of the anterior remnant of the preauditory placode is somewhat peculiar. It never extends beyond the hyoid gill pocket in any stage. The region just posterior to the gill pocket is where the proliferation of cells takes place to form the epibranchial ganglion of the VIIth nerve. This epibranchial placode, as will be shown later, begins as a thickening of the epidermis differing somewhat in appearance from the disappearing preauditory placode; but it is difficult to determine the exact relation of the last trace of the preauditory placode to the first trace of the epibranchial placode arising in the same area. A valid reason for considering the disappearing preauditory placode as distinct from the early stages of the epibranchial placode of the VIIth nerve lies in the difference in histological character of the two structures. The last stage of the preauditory placode in which it can still be recognized as such has elongated cells with nuclei placed on the inner border and shows no mitotic figures, while the early stages of the epibranchial placode has its cells irregularly arranged and mitotic figures in all stages are very frequent. Fig. 18 from the same embryo as that from which fig. 17 is taken lies six sections anterior to fig. 17 and two sections from the posterior end of the point of contact of the hyoid pocket with the epidermis, the contact of the pocket with the epidermis extending over eleven sections. The resemblance to the placode is still noticeable, the cells being elongated and, except at the central portion being only one cell deep. There are no mitotic figures present at this stage. This figure represents the last recognizeable trace of the preauditory placode.

THE ORIGIN OF THE GENICULATE GANGLION

Owing to the fact that both in point of time and in position there is such a close relation between the epibranchial ganglion of the VIIth nerve and the disappearing preauditory placode and that the cells derived from the epibranchial placode combine with cells from the lateral mass to form the definitive communis or geniculate ganglion of the VIIth nerve, it will be more convenient to describe the origin of the geniculate ganglion here than to follow the natural order and take up the differentiation of the post-auditory lateral mass. The series of figures (19 to 24, Stage VI) is taken from an embryo slightly older than the preceding, and in it the last trace of the preauditory placode has disappeared and the thickening which later gives rise to the epibranchial VIIth is just forming. Fig. 19 is taken at the point of contact of the hyoid endodermal pocket, with the epidermis and corresponds in position to fig. 18. It differs in two important respects; the epidermis dorsal and ventral to the contact with the endoderm shows no resemblance to the preauditory placode but is composed of cells whose outlines are very indistinct and whose nuclei are irregularly arranged. In addition to the disappearance of the placode in the region just mentioned, at the point of contact with the endoderm, the epidermis is closely fused with it and presents the appearance of being about to disappear entirely. This gill slit does not open completely in any of my series but the two layers of endoderm do separate in one of them, presenting the exact appearance of an open gill pocket. Fig. 20 is taken four sections posterior to fig. 19 at the point where the contact of the endoderm with the epidermis ceases and it is characterized by the irregular arrangement of its epidermal nuclei, some of which appear to have been proliferated mesially but are still in contact by cytoplasmic strands with the epidermis. Figs. 20, 21, 22, 23 are consecutive sections and lie just behind the point of contact of the gill pocket with the epidermis. In figs. 21 and 22 a proliferated mass of cells, quite small, is isolated slightly from the epidermis. The earliest stage of the epibranchial placode of the VIIth nerve is indicated by the thickened epidermis shown in these figures.

Tracing the placode back in a number of series leaves no doubt that this is the primordium of the placode. The manner in which this thickening of the epidermis increases in size and finally becomes detached, forming a portion of the geniculate ganglion, is quite easy to follow. Of the fate of the small mass of cells (*x*) shown in figs. 21 and 22, I am not certain. They may enter into the ganglion, but it is more probable that they become converted into mesectoderm. They lie a little anterior to the point of origin of the ganglion, and there is in some of my later series a small mass of cells anterior to the point of contact of the gill pocket with the epidermis, which resembles these, but their origin and fate I cannot determine definitely. If the cells shown enter into the ganglion, it is by attaching themselves later to the main ganglionic mass derived from the placode. Of this, however, I have no evidence.

Figs. 22 and 23 show a typical appearance of the early stage of any epibranchial placode and resemble closely the first stages of the placodes of the IXth and Xth epibranchial ganglia. The nuclei are several layers deep and quite irregular in arrangement and mitotic figures are beginning to be quite numerous. Fig. 24 is taken four sections posterior to fig. 23 and shows the transition of the placode into ordinary epidermis. Here, however, mitotic figures are still rather numerous but two sections posterior to this the epidermis is unmodified.

A comparison of the embryo from which figs. 19 to 24 (A. nebulosus, Stage VI) were taken with the one from which figs. 17 and and 18 (Stage V) were taken leaves little doubt that, while the epibranchial placode appears practically in the place where the preauditory placode disappeared, there is no direct relation between the two histologically. All of the preauditory placode posterior to the hyoid gill pocket is converted into mesectoderm and, while there is no evidence that the placode at the point of contact is ever converted into mesectoderm beyond the presence of the small mass of cells mentioned above, the fact that a contact with endoderm is formed would obscure the conditions here somewhat. While it is impossible to say that none of the cells that were once a part of the preauditory placode or their direct descendants enter

into the primordium of the epibranchial placode, the histological differences of the two structures and the fact that the one differentiates after the other has lost its characteristic structure show that the relation of the two placodes is more apparent than real. Added to this we have the evidence to be shown later that there is absolutely no relation between the postauditory placode and the epibranchial placodes of the IXth and Xth nerves.

The condition of the epibranchial placode in a somewhat older embryo in which the placode is still in contact with the epidermis is shown in figs. 25 to 30 (A. nebulosus, Stage VII). Fig. 25 is taken just posterior to the point of most intimate contact of the hyoid gill pocket with the epidermis. In this series the contact of the hyoid pocket with the epidermis, similar to that shown in fig. 19, (Stage VI) occupies only one section, while in the series from which fig. 19 was taken the contact is four sections in length. The hyoid pocket after coming into intimate relation with the epidermis gradually withdraws, and in the next stage following that from which fig. 25 was taken no longer reaches the epidermis at all.

Fig. 25 is taken at the extreme anterior end of the placode and the placode here differs from the epidermis anterior to it only in being somewhat thicker and in having its nuclei irregularly arranged in more than one row.

Fig. 26 is taken from the next section posterior to that from which fig. 25 was taken and there is here a decided thickening of the epidermis with numerous mitotic figures in various stages. In the next section (fig. 27) the thickening projects mesially as a well defined mass of cells and there are no less than twelve cells in various stages of mitosis in the placodal region of the epidermis. In the succeeding section (fig. 28) the proliferated mass of cells, the anterior portion of the future epibranchial ganglion, is larger and contains mitotic figures in addition to those in the epidermis. The ganglion is still attached to the epidermis in all these areas by a pedicle fully as thick as the ganglion itself. Two sections posterior to this point (fig. 29) the ganglionic proliferation changes somewhat in appearance, being longer and its attachment involving more of the epidermis ventral to it, so that

it passes gradually into the epidermis here, while on its dorsal surface it passes suddenly into rather thin epidermis. The appearance in this section indicates that the cells contributed to the ganglion before its detachment come mainly from the epidermis situated ventrally to its point of attachment. This conclusion is verified from a study of the epibranchial placodes of the IXth and Xth ganglia. The whole of the ganglion shown in fig. 29 is not derived from the placode. The portion situated mesially and dorsally (*L. M. G. VII*, fig. 29) is derived from the remains of the lateral mass lying anterior to the lateralis VIIth ganglion. The separation between these two constituents of the ganglion is faintly indicated in the figure. The presence of these lateral mass cells can be first detected in the section preceding the one from which fig. 29 is drawn and the separation is there somewhat more distinct than in the section sketched. In the section following the one from which fig. 29 was taken (fig. 30) the division between the two constituents is quite apparent and the portion of the ganglion derived from the lateral mass is slightly larger than the placodal constituent. A few sections posterior to this point the placodal portion of the ganglion ceases to be present, while the lateral mass portion reaches its maximum size in this embryo.

The posterior end of the lateral mass portion of the ganglion cannot be definitely determined, since at this stage it simply becomes looser in texture and finally before reaching the region of the lateralis VIIth can no longer be distinguished from that ganglion and from the ventrally situated mesoderm. The fact that this ganglionic mass is at its posterior end rather closely applied to the ventrally situated mesoderm makes it impossible to determine the exact boundary posteriorly before the ganglion assumes definite shape, as it does a little later after the detachment of the placodal portion; in addition to this fact the fibrillated root of the whole ganglionic mass appears at the posterior end of the ganglion and the point where this appears is in most of the cranial ganglia preceded by a more or less ill-defined mass of cells which renders the determination of exact boundaries difficult.

At this time the future ganglion consists of two constituents, first a placodal constituent proliferated from the thickened epidermis or epibranchial placode which is largest in its middle region and becomes smaller both posteriorly and anteriorly and is everywhere still attached to the epidermis although it later becomes completely detached. Secondly there is a lateral mass constituent lying on the dorso-mesial portion of the placodal constituent and ending posteriorly in loose lateral mass cells so that at this stage its posterior boundary cannot be definitely determined.

As the geniculate ganglion becomes larger the difference in size between the body of the ganglion and its root enables one to determine approximately the posterior extremity of the ganglion. This can be done with certainty only when the fibrillated root appears. At this time the trigemino-facial complex has the arrangement indicated in fig. 83 when the Gasserian and geniculate ganglia overlap for about one-half the length of the Gasserian. This overlapping is due apparently mainly to the forward growth of the geniculate on the mesial side of the Gasserian, the root of the Gasserian remaining constant in position and the body of the ganglion extending only slightly posterior to its original position. The forward extension of the geniculate is probably entirely due to growth in size of the ganglion, since I can find no evidence of the further proliferation of cells anterior to the point occupied by the placode.

Both the Gasserian and geniculate ganglia assume a definite form with well defined boundaries rather slowly, so that for some time they consist of condensed masses of cells with irregular outline. Since the extent of fusion of the acustico-facial complex is very great in the adult in Ameiurus, I have given a series of figures (31 to 39) to show the condition of the ganglia at a time when all the components are still separate and the roots and chief trunks of the nerves derived from these ganglia are still distinct. These figures are taken from the same embryo (A. melas 86 hours) as that from which fig. 83 was constructed. This plot should be compared with fig. 2 of Herrick's paper ('01). The principal point of interest here lies in the fact that the roots and chief

trunks derived from these ganglia are found to contain only general cutaneous or communis or lateralis fibres.

Fig. 31 is taken through the origin of the trunk of the Gasserian ganglion, and fig. 32 just anterior to the point where the trunk of the geniculate ganglion leaves the ganglion, and consequently lies between the trunks of the Gasserian and geniculate ganglia. These two trunks are entirely separate not only at their point of origin but throughout their whole course at this time. They form the supero-lateral and infero-mesial strands respectively of Wright ('84*b*). These combine and later separate to give rise to the maxillary and mandibular trunks which are mixed, containing general cutaneous, visceral, and lateralis components. Herrick ('01, p. 183) could not determine positively that these two strands were pure but there can be no doubt that they are pure at this stage and that the supero-lateral strand contains fibres from the Gasserian ganglion only and that the infero-mesial strand contains fibres from the geniculate ganglion only. Further, the roots are not yet in contact at any point nor do they contain lateralis fibres as yet.

Figs. 33 and 34 are taken through the root of the Gasserian ganglion and show that the root is no less distinct than the trunk. The dorso-lateral lateralis VIIth also appears in these sections. The roots of the geniculate and Gasserian ganglia are separated by 15 sections, while the trunks at this stage are six sections apart. The overlapping of the ganglia finally brings the trunks into the same plane but even then there can be no doubt that they are derived separately from their respective ganglia.

Fig. 35 is taken through the origin of the hyomandibular nerve (*T. V. L. VII*) which is at this time a pure lateralis nerve derived from the ventral lateralis VIIth ganglion (*V. L. VII*). This nerve later contains in the adult small strands of general cutaneous and visceral fibres (Herrick, '01) but is at first a pure lateralis nerve. The ramus oph. sup. VII is in the adult a pure lateralis nerve. In this stage it can be detected as a forward extension of the anterior end of the dorsal lateralis VIIth ganglion but it is only faintly fibrillated. The ramus oph. sup. V, I cannot detect at this stage but it is probably mixed at an early stage, since the

Gasserian and geniculate ganglia from which it derives its two components lie side by side and their anterior ends from which the nerve arises are intimately in contact when it can first be detected. The arrangement of the roots of the geniculate, lateralis VIIth and auditory ganglia are shown in figs. 36 to 39. They are all quite distinct and can be followed to the point of contact with the medulla.

As mentioned above, from this time on the components of the Vth and VIIth ganglionic complex become more intimately fused so that it is not surprising that nerve trunks which are at first undoubtedly pure trunks, *i.e.*, contain only one component, later become mixed and contain two or more components. The contiguity of the ganglionic masses furnishes a basis for the mixing. Those components which are not found in the nerve at first must grow into it later. They are unquestionably there in the adult and not there in the embryo. The geniculate ganglion wedged in between the Gasserian and lateralis VIIth ganglia is in a particularly favorable position to send its fibres into nerves derived primarily from other ganglia. This has gone so far in Ameiurus that 12 out of 14 of the chief nerves coming from the acusticofacialis complex contain visceral fibers, as compared with five in Gadus and Menidia.

There are, at least, three methods by which nerves become mixed. The first is illustrated by the maxillary and mandibular trunks which arise some distance from the ganglion, after the fusion of two pure strands, the supero-lateral which is pure general cutaneous and the infero-mesial which is pure visceral. These pure strands after coming into contact break up into two mixed nerves, the maxillary and the mandibular. A second method by which nerve trunks become mixed is illustrated by the hyomandibular which is at first a pure lateralis nerve but later becomes mixed by having general cutaneous and visceral fibres grow into it from their respective ganglia. A third method is illustrated by the rami oph. sup. V and VII which, owing to the contiguity of its ganglia, the Gasserian and geniculate, seems to be mixed from the first, the roots growing out from the ganglia in contact with each other.

DIFFERENTIATION OF THE POSTAUDITORY LATERALIS MASS

The changes which take place in the lateral mass posterior to the ear resemble in a general way those which take place anterior to the ear. The postauditory lateral mass gives rise to a post auditory placode situated ventro-laterally, continuous with the auditory vesicle, while the dorso-mesial region breaks down into a loose mass of cells which is converted partly into mesectoderm and partly into the general cutaneous and general visceral ganglia of the Xth nerve. As mentioned above, the lateral mass is at first entirely homogeneous. Fig. 3 represents the appearance throughout its whole extent at an early stage, except that the lateral thickening is slightly less marked as it passes into the region of the spinal cord.

The first evidence of differentiation posterior to the ear is shown in fig. 5 (A. nebulosus, Stage III) which is taken four sections posterior to the posterior end of the auditory vesicle. There is noticeable here a slight distinction between the ventro-laterally situated postauditory placode and the dorso-mesial region. The separation between these regions is indicated by the mode of contact of the cell walls on either side of the dividing line and by the appearance of a vacuole. This sketch is taken from the same embryo as that from which figs. 4, 6, 7, 8 and 9 were taken. The differentiation of the preauditory lateral mass is only slightly in advance of that of the postauditory mass. The changes in the dorso-mesial portion of the postauditory mass by which it becomes converted into mesectoderm resemble closely those that take place in the region of the Gasserian ganglion. On either side of the Xth ganglion the dorso-mesial portion of the lateral mass becomes converted into a very loose mass of mesectoderm, except at its ventral border; here where it comes into contact with the mesoderm it remains slightly denser.

In the region where the Xth ganglion forms and possibly where the IXth forms the process of conversion into mesectoderm does not go so far and the lateral mass in these regions is in some embryos slightly denser than in the surrounding regions. These two regions however are only slightly denser and, in a stage fol-

lowing the breaking down of the dorso-mesial portion of the lateral mass present the appearance of not having broken down so completely as surrounding areas. A comparison of figs. 40 to 43, (A. nebulosus, Stage VII) all from the same embryo, brings out these relations in an embryo in which the ganglionic regions are unusually well marked. Fig. 40 is taken one section posterior the auditory vesicle. The whole region between the cord and the endoderm is occupied by the derivatives of the lateral mass. Just dorsal to the endoderm (fig. 40, *Y*) the tissue is somewhat dense and there is also a slight condensation (fig. 40, *G*) at the level of the upper third of the medulla; this condensation extends into the next section (fig. 41, *G*) but disappears in the section following that from which fig. 41 was taken. From this point back to the region where the Xth ganglion appears the lateral mass presents the appearance shown in fig. 42. The mesectoderm presents the appearance ofmesenchyme having well defined nuclei but with ill-defined cytoplasmic branches forming a loose network. Just over the mesoderm the derivatives of the lateral mass in all three figures are somewhat denser. It will be recalled (pp. 330–331, 345) that it is this portion of the lateral mass that forms the Gasserian and geniculate ganglia in the preauditory region.

Figure 43 is taken through the region of the Xth ganglion just anterior to the anterior end of the lateralis Xth. This section is taken just back of the anterior end of the ganglionic mass (*J. G. X*, fig. 43). The anterior end of the ganglion lies somewhat nearer the medulla than in the section figured, while posterior to this point the mass is found more ventral and lateral in position, finally coming into contact with the denser portion indicated in figs. 41 and 42, (*Y*), lying just over the mesoderm. The loose mass of cells shown in fig. 43 (*J. G. X*) gives rise to the jugular ganglion to be described later.

Like the early stages of the Gasserian ganglion, the whole mass is extremely ill-defined with irregular borders passing gradually into the surrounding mesectoderm. The ganglionic mass does not reach the dorsal portion of the medulla and there is no indication of a migration of cells from that structure.

At this stage in the formation of the ganglion there is nothing to indicate its presence except the slightly denser massing of the cells and the somewhat denser character of the inter-nuclear material. There is one rather striking difference between the Xth and preauditory ganglia. Anterior to the ear all the ganglia trend down and forward from their points of attachment to the brain where the fibrillated roots will appear. The Xth ganglion derived from the lateral mass in the same manner trends down and back from the point where its fibrillated root will appear, reversing the relations to the medulla. These figures are taken from an embryo in which the condensations in the region of the IXth and Xth ganglia are usually well marked. From this stage up to the seventy-fifth hour in A. melas there is no well defined ganglion even in the region of the Xth, and in several series I can find no evidence of either ganglion. Between the 49-hour and the 56-hour embryo, A. melas, there is nothing that can be identified positively as a Xth ganglion, although one can be quite sure of the region in which it ought to be located. In embryos of 61 to 65 hours, A. melas, there are slight condensations in the region of the Xth, but they would hardly be detected if one did not read back from older series. The ventral derivative of the lateral mass (*Y*, figs. 41, 42) is present in the region of the Xth during this time, however, and it is this region which furnishes the greater portion of the visceral Xth. During the whole time under discussion the lateralis Xth ganglion is present and furnishes a good landmark in the attempt to locate the early stages of the visceral Xth.

By the seventy-fifth hour (A. melas), the ventral portion of the Xth ganglion derived from the lateral mass has assumed a definite form and can be followed from this time on easily. It occupies at this stage a ventro-mesial and mesial position with reference to the lateralis Xth which is an elongated cylindrical mass of cells with quite definite boundaries. The visceral Xth extends from near the anterior boundary of the lateralis Xth to a point beyond its middle portion and the motor root of the Xth extends along the mesial side of the lateralis Xth running forward and upward to the medulla. At this stage the lateral mass ganglion of the Xth is

situated low in the body on a level with the dorsal portion of the gill slit and is connected with the medulla by the narrow strand of loose cells described above. The outline of the ganglion is still indefinite and its ventral portion is closely applied to the mesoderm underlying it. All of the visceral portion of the Xth except that derived from the placodes seems to come from the ventral portion of the lateral mass, while the jugular or general cutaneous ganglion comes from the dorsal portion. The jugular ganglion cannot be detected for some time after the visceral Xth is formed.

Owing to the intimate relation of the lateral mass portion of the Xth ganglion to the epibranchial placodes, it will be better to describe the lateralis Xth first and return to the visceral Xth later (p. 79). The fate of the lateral mass cells in the region of the IX ganglion will be taken up with the lateralis IXth. The relation of the visceral Xth ganglion to the jugular or general cutaneous Xth which appears later and is situated near the medulla intracranially, is extremely interesting. It will be recalled that the lateral mass anterior to the ear gives rise to the Gasserian, a pure cutaneous ganglion, and to a part of the geniculate, a visceral ganglion which fuses with the ganglion from the epibranchial placode. Both these ganglia become closely fused in the adult and are situated intra-cranially. In the Xth, however, we have a complete separation of the general cutaneous and visceral portions; the former being small and situated intra-cranially, the latter large and situated extra-cranially and fusing with the placodal ganglion just as the geniculate does. From evidence to be brought out later it appears that the lateral mass contingent of the geniculate and visceral Xth is really a general visceral component, the special visceral or gustatory ganglia being derived from the placodes. In types having a well defined neural crest these same relations would seem to hold, *i.e.*, the general cutaneous and general visceral ganglia of the head have a common derivation from the neural crest, which is homologous with the neural crest of the spinal cord. The chief difference between the brain and the cord in this respect lies in the greater size of the cranial ganglia and the extent to which the general cutaneous component and general visceral component, which are both represented in the spinal ganglia,

are separated in the head. In the more generalized Xth the general cutaneous is intra-cranial and the general visceral extra-cranial, while in the more highly specialized region of the Vth and VIIth both are intra-cranial.

THE ORIGIN OF THE LATERALIS XTH AND THE EARLY STAGES OF THE POSTAUDITORY PLACODE

The lateralis Xth is derived not from the lateral mass but from the postauditory placode as it moves away from the auditory vesicle. The first evidence I find of a separation between the postauditory placode and the auditory vesicle is shown in fig. 44, Stage IV. The dorsal portion of the auditory pit was present in the preceding section. In fig. 44 only the ventral portion remains as the placode. The portion which disappears is that most nearly in contact with the medulla. In the following section (fig. 45) the placode has lost all connection with the dorso-mesial portion of the lateral mass which is beginning to be converted into mesectoderm. The length of the placode at this time is only four sections. Fig. 46, Stage IV, shows the appearance of the placode in a series of the same age, but less developed, in which the auditory vesicle is continued into the placode with no break in continuity. The placode is here seven sections long.

The placode now moves back apparently after it is detached from the auditory vesicle. My series is incomplete at this period and I am consequently unable to describe the earliest appearance of the anterior end of the lateralis Xth or the rate at which the placode moves away from the auditory vesicle at first. At the time, however, when the anterior end of the placode has reached a distance of twenty-one sections from the vesicle, Stage VII, the placode has not changed in appearance but the lateralis Xth ganglion is present and has a length of ten sections and overlaps the placode for five sections. Anterior to the region of overlap the lateralis ganglion lies just under the epidermis between it and the mesectoderm as a rather irregular mass of cells (fig. 47). Throughout the whole region of the overlap the placode is contributing

cells to the ganglion. As one reads from the anterior towards the posterior end of the placode, the placode gradually becomes larger until we reach the middle region (fig. 50); it then gradually diminishes in size until it assumes the appearance of the ordinary epidermis. Coincident with the increase in size of the placode in its anterior portion there is a decrease in the number of cells in the overlapping ganglion (figs. 48, 49, 50) until just posterior to the middle (fig. 51) there are no ganglion cells present. This condition is duplicated in all my series in which the ganglion and placode overlap.

In an embryo of 56½ hours (A. melas) the placode has moved beyond the posterior end of the ganglion and there is a space of three sections between the placode and the ganglion.

From this time on the placode moves steadily back from the region of the ganglion and as it moves away gradually loses its resemblance to the earlier condition. Its cells cease to have a radial arrangement and it is recognizable simply as a thickening of the epidermis. The appearance of the ganglion anterior to the region of the overlap is shown in fig. 52 (Stage IX). The question as to whether the postauditory placode moves bodily away from the auditory vesicle or whether the placode at successive distances from the vesicle represents a localized differentiation at those points has been variously answered. The anterior end of the placode seems to be partly converted into ganglion cells and partly to revert to ordinary epidermis as far as its appearance is concerned. The posterior end of the placode seems to arise by the conversion of epidermal cells into placodal cells. There is no recognizable train of cells left in the epidermis in the route of the moving placode. The lateral line organs which appear long after the placode has passed a given point appear approximately along the route traveled by the placode, but are not derived from the placode.

The reason for thinking that the placode moves by successive differentiations and does not migrate bodily rests on the fact that in the region of the placode, part of the placode becomes detached as the lateralis Xth ganglion and the remainder not so used is detached or passes gradually into ordinary epidermis, since there is no abrupt transition from placode to epidermis. After

the placode ceases to contribute cells to the ganglion the ganglion ends bluntly posteriorly and there is no strand of cells connecting the ganglion and placode. The trunk of the lateral line nerve of the Xth grows out as a new structure from the ganglion.

THE FATE OF THE POSTAUDITORY PLACODE AND THE APPEARANCE OF THE LATERAL LINE ORGANS OF THE BODY

After the postauditory placode is no longer in contact with the lateralis Xth ganglion it moves back some distance from the ganglion and remains approximately stationary from the fifty-sixth hour up to the one hundred and thirteenth hour (A. melas), during which time it gradually becomes smaller and less distinct. During the greater portion of this time it can be identified positively both by its appearance and by its position in the body and by the fact that there is nothing else with which to confuse it. In the mean time there have appeared in the region traversed by this placode in its backward movement and in the region from which the auditory vesicle was detached from the ectoderm four lateral line organs. These are the first four described by Herrick ('01, plate xiv, fig. 1). The first according to his nomenclature, which is innervated by the ramus oticus from the VIIth nerve, arises lateral to the anterior end of the auditory vesicle and appears first in my series in an embryo of 105 hours. The auditory vesicle at this time is beginning to form the semicircular canals. The second organ, innervated by the ramus supratemporalis IXth, develops between the posterior end of the auditory vesicle and the anterior end of the lateralis Xth ganglion. It appears first in the embryo of 105 hours. The third and fourth organs innervated by twigs from the lateralis Xth ganglion develop in close proximity to the posterior end of that ganglion from a common primordium which elongates and gives rise to two organs. The anterior portion of the common primordium gives rise to the third organ and the posterior to the fourth organ. This common primordium of the third and fourth organs appears first in an embryo of 86 hours and about nineteen hours before the appearance of the primordia of the first and second organs.

At the time of the appearance of the common primordium of the third and fourth organs the postauditory placode lies about five sections posterior to it and can be distinguished easily from the primordium by the fact that the placode still retains its characteristic appearance, having elongated radially arranged cells, while the primordium bears no resemblance to a lateral line organ, being merely a thickening of the epidermis. Here, as in all other lateral line organs, one must first locate the organ after it is fully formed and then read back in series and locate the primordium. This can be done first by counting sections and second by using convenient land-marks in the body. This is necessary because none of the lateral line organs at first have any resemblance to the adult organs.

In an embryo of 99 hours (A. melas) while the postauditory placode is still recognizable as a thickening of the epidermis there have appeared at least two lateral line organs posterior to it. These two organs can be traced continuously in my older sections and lie posterior to the large branch of the Xth nerve, while the placode lies anterior to it. Owing to the fact that structures frequently do not develop uniformily even when a series of graded ages is used, it is not possible to make definite statements in regard to the last stage of the postauditory placode. Of its moving back from the vesicle and of its gradual reduction I am quite sure. In an embryo of ninety-nine hours (A. melas) while the third and fourth lateral line organs are still contained in the common primordium the placode is recognizable and back of this are two primordia of lateral line organs. In an embryo of one hundred and five hours the placode is present but no organs lie posterior to it. In an embryo of one hundred and thirteen hours the two lateral line organs previously mentioned as lying posterior to the placode are present in practically the same position as in the embryo of ninety-nine hours, having moved slightly away from the vesicle while the sections occupied by the placode in the preceding series are vacant and remain vacant in later series. These two organs persist in my later series and I infer that the postauditory placode disappears posterior to the position of the

fourth lateral line organ and gives rise to no organs. If any do arise from it it would be the fifth of the body lateral line.

As to the mode of origin of the organs, there seems to be little variation. Each one appears as a slight thickening of the epidermis scarcely perceptible at first except by its location at the seat of the future organ. This thickening becomes more pronounced by the elongation of the deeper cells and by their assuming a radial arrangement with frequently a small circular cavity just beneath the outer flattened layer of epidermis and at the apex of the radially arranged cells. This process of the elongation of the radially arranged cells continues until the organ assumes its permanent form. The variation mentioned above consists in the different rates at which this process is carried on and also the different stages of development at which the organs sink into canals. The facts brought out above differ materially from the usual description of the relation of the dorso-lateral placode to the lateral line organs and lateral line nerve but are in close agreement with the work of Miss Platt on Necturus ('95). The fact that anterior to the ear the preauditory dorso-lateral placode disappears more than 26 hours before lateral line organs can be detected and that posterior to the ear at least four lateral line organs appear anterior to the placode, while it can still be recognized as such, leaves no doubt in the writer's mind that there is no evidence of a genetic relation between the dorso-lateral placodes and lateral line organs in Ameiurus. The lateral line organs are definite differentiations of the epidermis, just as are the taste buds, and the nerves which supply them grow from specific ganglia just as in the case of the gustatory nerves. There seems to be no doubt that the auditory vesicle and pre- and postauditory placodes are homologous, or rather the same structure, but the relationship of the lateral line organs and vesicle is on a somewhat different footing.

If the auditory vesicle phylogenetically is to be looked upon as containing sensory areas homologous to lateral line organs, in the ontogeny we have the troublesome fact that lateral line organs arise in the epidermis in the same area as that from which the vesicle arose; further than this the preauditory lateral line organs

are innervated from ganglia derived from the lateral mass or neural crest of other types while those posterior to the ear are innervated partly by ganglia derived from the auditory vesicle and partly by ganglia derived from the posterior extension of the vesicle or the postauditory placode.

The foregoing description of the relations existing between the pre- and postauditory placodes and the lateral line organs, differs so much from that of Wilson ('91, '97) in the sea bass and salmon that it merits a fuller discussion. The accounts differ as to the mode of origin of the lateral line organs, Wilson tracing them to the sensory lines derived from the postauditory placode and the preauditory placode (branchial sense organ of Wilson and Beard) while the present account traces them to differentiations of the epidermis not derived from the placodes. Wilson's statement of the case as given in his short paper on the salmon ('97) is as follows and will stand for the conception of those authors who agree with him. He states that in Serranus

> The organs of the lateral line, the auditory sac, and the superficial sense organs of the head (presumably all) were derived from a common foundation. This common foundation has the shape of a long furrow (ectodermic) on the side of the head region. The furrow splits into three parts, the posterior part giving rise by division to the organs of the lateral line, the middle part becoming the auditory sac, the anterior part becoming a histologically developed branchial sense organ, situated in front of the single gill slit of the embryo, from which a (sensory) cord of cells is prolonged forwards.

Wilson finds practically the same condition in the salmon except that the pre- and postauditory furrows are only thickenings in this form, and cites Mitrophanow ('93) and Locy ('95) as agreeing substantially with him.

The paper on the salmon is brief and does not describe the exact mode of appearance of the definitive lateral line organs. An examination of Wilson's paper on the sea bass ('91) shows that the relation of the preauditory lateral line organs to the preauditory placode is much less definite than in the paper on the salmon. He

says (p. 246) in discussing the fate of the preauditory placode (branchial sense organ) that "the further development of the organ consists in the loss of its cavity, in histological differentiation, and in the transformation of its ill-defined anterior extremity into two cellular cords which doubtless serve as source for the production of new organs." On the following page in discussing the anterior sensory tract he states that "during larval life one or two sense organs are found in this region and it is extremely probable that they arise from the dorso-lateral tract." In the following paragraph is the statement that "the anterior sensory tract is at the time of hatching very short and just what becomes of it I do not know." This anterior sensory tract is the tract which Wilson derives from the anterior end of the branchial sense organ and which gives rise by its forward extension to two tracts.

It may be well to call attention to the fact that where Wilson makes unequivocal statements in regard to the relation of the auditory vesicle to the preauditory placode, and to the postauditory placode in part, we are in essential agreement, *i.e.*, in the origin of the auditory vesicle and the pre- and postauditory placodes from a common primordium, the detachment of these placodes from the vesicle and in the disappearance of the preauditory placode. Wilson finds that the preauditory placode disappears, while the postauditory continues to grow back giving rise to the lateral line, but I find that both the pre- and postauditory placodes finally disappear. With this exception up to this point the two accounts are quite similar. The presence of sensory cells in Wilson's branchial sense organ and their absence in Ameiurus is not a radical difference, especially since the preauditory placode in Ameiurus has such a close resemblance to the auditory vesicle.

As to the point of difference, a careful reading of Wilson's paper fails to show a definite relation between the branchial sense organ and the anterior sensory ridges, and the specific lateral line organs. The disappearance of the branchial sense organ and the anterior sensory tract, and the appearance of specific

lateral line organs in the same region is not positive evidence of genetic relationship between the two structures. The divergence in our accounts begins in the description of the method by which the specific lateral organs arise, more particularly as to whether the sensory ridge on which Wilson finds the lateral line organs differentiating is an extension from the pre- and postauditory placodes. In Ameiurus I find no common sensory ridge from which the organs differentiate, and can trace the gradual disappearance of the pre- and postauditory placodes. Miss Clapp ('99, pp. 239 251) states that these sensory ridges originate in the auditory region but nowhere I think traces them to the auditory vesicle or its derivatives, while Miss Beckwith ('07) states definitely in tracing the genesis of the lines that they do not come from the auditory vesicle but arise as local differentiations of the epidermis on which the lateral line organs later appear (p. 28).

There is a bare possibility that the relation of the anterior sensory ridge to the branchial sense organ as described by Wilson in Serranus is one of contiguity only and not of genetic relationship. If this be admitted, and it is the point about which Wilson is least definite, then there are no insuperable difficulties in harmonizing the two views, for the lines seen by Allis ('89) and other workers may be interpreted in two different ways. They might be continuous ridges which break up into individual sense organs, or on the other hand they might be formed by a series of individual sense organ primordia whose extremities become confluent. There is evidence for both views. Wilson is very postitive concerning the mode of formation of the postauditory lateral line organs from a common primordium derived from the postauditory extension of the vesicle in the sea bass, while I have evidence which I do not think can be doubted that even the postauditory lateral line organs arise separately and have nothing at all to do with the postauditory placode. This conclusion was reached by Hoffmann ('94) and by Miss Platt ('95, '96) in part of the organs in Necturus. The same conclusion is reached by Miss Beckwith in Amia. Allis's work ('89) does not really confirm Wilson, since his earliest stage was about one day old (after hatching) and this is too late to

trace the genesis of the sensory lines. Miss Beckwith shows that the lines from which the lateral line organs appear are entirely distinct from the auditory vesicle and that the supra-orbital, sub-orbital and mandibular sensory ridges arise separately. Locy states that he agrees with Wilson but gives no description of the origin of the specific lateral line organs. It will serve to clear the ground for future work if it is remembered that there are two distinct problems here; first, do the pre- and postauditory lateral line primordia grow out from the auditory vesicle or its extensions the pre- and postauditory placodes, and, second, do the lateral line organs appear individually or do they differentiate from a common primordium?

Admitting that the lateral line organs do differentiate in some types from common sensory ridges and in other types as individual organs, the question arises as to which is the more primitive method. If the appearance of individual organs as in Ameiurus, each of which is homologous with sensory areas of the auditory vesicle, is the primitive method, it is conceivable that the elongation and precocious appearance of the primordia from which these organs appear (and these primordia are always longer than the organs in Ameiurus) might result in a more or less definite line such as Wilson describes in Serranus and Miss Clapp in Batrachus. The growth of the postauditory sensory line would mean nothing more than the appearance of successive organs whose primordia have become continuous. If the differentiation of organs on lines which are extensions of the auditory vesicle is the primitive mode, it is more difficult to understand how the method by which they arise in Ameiurus could be derived from it. It is hard to see how organs that primitively come from a common primordium could later in phylogeny arise singly. The difficulty is increased when we remember that the lateralis VIIth ganglion does not come from the vesicle or any of its derivatives but from the lateral mass (neural crest). Perhaps the greatest obstacle to this view on theoretical grounds arises from the confusion which is introduced into current ideas of the primitive relation of the lateral line organs to the sensory areas of the audi-

tory vesicle. If these organs arise as differentiations of sensory ridges which grow out from the anterior and posterior extension of the auditory vesicle, they cannot be strictly homologous with the sensory areas of the auditory vesicle, since they are derived from it, unless we consider the ear as precociously developed on this ridge. If on the other hand the lateral line organs arose primitively as single organs, the sensory areas of the auditory vesicle and lateral line organs can be considered as homologous and the conditions in Serranus, Salmo, Batrachus, and Amia can be explained as modifications of this primitive method.

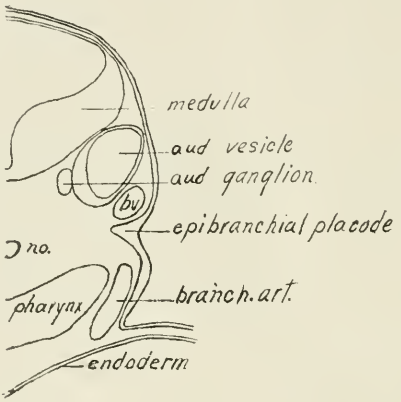
THE PLACODAL GANGLION OF THE IXth NERVE

The visceral ganglion of the IXth nerve stands in a rather unique position since it has no recognizable constituent derived from the lateral mass, and is therefore a pure placodal ganglion; otherwise it presents many points in common with the placodal ganglion of the VIIth and those of the Xth to be described later. It begins as a thickening of the epidermis on a level with the dorsal limit of the first true gill slit, and extends posteriorly from this point. The thickening is characterized by the irregular arrangement of its nuclei, and by the number of mitotic figures. The stage of thickening is followed by a process of proliferating cells mesially and posteriorly, and this group of proliferated cells is finally detached *en masse* and acquires a connection with the more dorsally situated lateralis ganglion. Fig. 53 from a 56½-hour embryo (A. melas) is taken just posterior to the point of contact of the visceral pocket with the epidermis. The placode is recognizable at this stage as a simple thickening of the epidermis extending from the level of the dorsal border of the future gill slit down to the level of the branchial artery and longitudinally occupying three or four sections. The epidermis is several cells thick and usually, especially during later stages, shows many mitotic figures. The epidermis is nearly always slightly indented at the point where the placode appears, but whether this is to be associated with the placode or with the gill slit is not clear. The placode differs structurally from the epidermis adjoining it in the

characters mentioned, and in addition it takes a slightly darker stain and its inner border is usually less ragged than that of the surrounding epidermis. The epidermis anterior and dorsal to the placode rarely has a well defined border on its inner surface, but presents the appearance of proliferating cells into the mesectoderm. The placode passes almost imperceptibly at first on its anterior and posterior borders into the epidermis. On the dorsal surface, however, the transition from placode to epidermis is quite sudden. In the region of the placode, however a well defined mesial border can be seen and, while it could not be said that no cells slip individually from this placode into the mesectoderm, there is no evidence of it.

This process of thickening of the ectoderm is followed a few hours later by a movement of the cells *en masse* into the region of the mesectoderm in a posterior and dorsal direction. Fig. 54, taken at the middle of the placode, shows its appearance in an embryo of 69 hours. It occupies about the same relative position as in fig. 53, but the auditory vesicle which was absent in fig. 53 appears in this section (not shown in the figure, however), owing apparently to its extension backward. The placode has a much denser appearance here than the surrounding mesectoderm and its boundaries can usually be located with no difficulty except at the posterior end which is ill defined, except in the earliest stages before the placode begins to project mesially. The placode is shorter dorso-ventrally than in the preceding stage, and on the side from which the sketch was made not more than four sections long, but on the opposite side as much as seven sections long. On its ventral, anterior, and posterior borders it passes over into ordinary epithelium, as in the preceding stage described. Its mesial projection at the thickest part from which fig. 54 is taken is about three times the thickness of the adjoining epidermis. Cell multiplication is taking place as evidenced by the presence of mitotic figures; in fact, there is evidence of both cell migration from the epidermis after the placode begins to project mesially, and of the development of cells *in situ* in the projecting portion of the placode.

In the next series, 75 hours, which I have not figured, little change has taken place, although the placode extends somewhat farther mesially; but in an embryo of 81 hours (fig. 55) a change can be noticed. Figs. 55, 56, 57 are consecutive figures taken from the same embryo. Fig. 55 is taken from the anterior portion of the placode and shows a decided constriction between the ganglionic mass and the epidermis with which it is still attached. The epidermis immediately dorsal to the attachment of the placodal ganglion has resumed its normal appearance, having a rough



E

Fig. E. A camera tracing corresponding to fig. 56, showing the position of the epibranchial placode. NO. notochord; b.v. blood vessel.

inner border composed of cells which seem to be in process of becoming mesectoderm. Ventral to the placode the epidermis is still thick and the appearance here, as elsewhere in the formation of epibranchial placodes, indicates that the epidermis ventral to the ganglion is the chief source of whatever cells migrate into it.

The following section (fig. 56) presents approximately the same appearance except that the nuclei of the epidermis are separated from those of the ganglion which is attached to the epidermis by a cytoplasmic mass only. In fig. 57, however, we find the ganglionic mass detached from the ectoderm and lying free in the mesectoderm, while the epidermis resembles that of adjoining

regions. This portion of the ganglion was never attached to the epidermis at this point, but has grown backward and upward from the points indicated in the two preceding figures. The ganglion has now become longer than its point of attachment to the epidermis. Fig. 60, 93 hours, illustrates the first stage in which the epibranchial ganglion of the IXth is first completely detached from the epidermis. This section is taken through the point where the ganglion lies nearest the epidermis, *i.e.*, near the anterior border of the ganglion. Careful measurements of the length of the ganglion and of its point of attachment in a series of stages make it evident that the ganglion grows as indicated.

TABLE III

Showing the relative length of the epibranchial ganglion of the IXth as compared with the length of attachment (A. melas).

Age in hours	69	75	81	86	93	99	105	113
Length of attachment to placode in sections	3	2	2	2	0	2	1	0
Length of epibranchial ganglion in sections	3	7	12	12	19	25	13	15

In the first series (69 hours) the mesially projecting mass is no longer than the thickening, but in the following series the ganglion occupies seven sections and the attachment two. In the later stages the ganglion elongates rapidly up to 99 hours, where it is twenty-five sections in length, and after that, owing to its becoming placed in a dorso-ventral position, is not so long. In an embryo of 93 hours the ganglion is not in contact with the skin, but is found in contact in two later series, 99 and 105 hours, and after 113 hours it is permanently detached. The placode does not present the appearance of adding cells to the ganglion after the 93-hour series. The anterior end of the ganglion seems to be simply lying in contact with the epidermis which no longer resembles a placode. The epidermis has not yet resumed its normal appearance, but shortly after this stage there is nothing in the character of the epidermis to indicate the point at which the ganglion arose.

The introduction of the term 'branchial sense organ' by Froriep and Beard seems unfortunate. Froriep ('85) applied it to the epibranchial placodes of mammals, and Beard ('85–'86) to both epibranchial and dorso-lateral placodes apparently, and the term is used by Wilson ('91) in his paper on the sea bass. In all cases the term seems an unfortunate one. The epibranchial placodes of Ameiurus have no resemblance whatever to sense organs, particularly to gustatory organs, and the dorso-lateral placodes while resembling, in Ameiurus, lateral line organs do not give rise to lateral line organs. This is true in Necturus, as shown by Miss Platt ('95). The branchial sense organs of Wilson are apparently the dorso-lateral placodes. Whatever may have been the phylogenetic history of these placodes, it is much better to designate them in such a way as not to commit oneself to a theory as to their phylogenetic origin until it can be shown what that origin was. I have, therefore, followed von Kupffer ('94) and have used the term 'placode' exclusively. The distinction brought out (p. 56–57) in the discussion of the relation of the pre- and post-auditory placodes to the specific lateral line organs finds a striking parallel in the relations of the epibranchial placodes to their specific sense organs, the taste buds.

The lateral line organs arise in Ameiurus as definitely localized differentiations of the epidermis, and are not derived from the dorso-lateral placodes (pre- and postauditory placodes of Ameiurus), although they receive their innervation from ganglia derived from these placodes in the case of the X, IX and the VIII nerves.

The taste buds bear the same relation to the epibranchial placodes, as shown by the writer (Landacre '07). The taste buds appear simultaneously at the extreme anterior end of the oral cavity (ectoderm) and on the endoderm of the first three gill arches and spread posteriorally from the gills into the pharynx and œsophagus and from the anterior end of the oral cavity back into the mouth and externally over the lips, barbules and outer surface of the head and finally over the whole body.

The taste buds appear in well defined groups determined largely by the distribution of the rami of the nerves carrying gustatory fibres. These groups are isolated at first and later become con-

fluent and always appear in order from anterior to posterior. The taste buds show one other peculiarity in their order of appearance. They appear, generally, at the peripheral distribution of the nerves innervating them anterior to the ear, and in the reverse order posterior to the ear. It is evident from these facts that they are in no way, in Ameiurus, closely related in place of origin to the epibranchial placodes. The epibranchial placodes, so far as any evidence secured from their ontogeny indicates, are to be looked upon as ganglion forming structures. The epibranchial placodes bear no resemblance to either single taste buds or groups of taste buds. These buds spread in the case of the oral and superficial head buds from the point of origin toward, and not away from the point of origin of the placodes.

An additional fact of significance is the time intervening between the detachment of a given placode and the appearance of the first taste buds innervated by fibres derived from the ganglion formed by the placode. In the IXth nerve, the placode which forms the special visceral ganglion of that nerve becomes detached in a 93-hour embryo (A. melas), but the first taste buds on the pharynx, and, in fact, on any part of embryo, appear in a 113-hour embryo; a length of time sufficient to suggest tht the appearance of the taste buds is associated with the appearance of the nerve trunk, rather than with the proximity of the taste buds to the placode, since the IXth nerve has a fibrillated root and trunk which extends into the first gill arch in an embryo on 113 hours.

The process of growth by which the various components of the peripheral nerves find their appropriate sense organs furnishes one of the most puzzling problems in embryology. This is especially true of the gustatory nerves and taste buds, and in a measure is true of the lateralis system of nerves and sense organs. The solution does not seem to lie in deriving both nerves and sense organs from a common primordium, as can be done in the case of the olfactory nerve and optic nerve. The distance between the point of origin of taste buds supplied by the VIIth nerve and the point of origin of the ganglion of the VIIth nerve precludes such an explanation. The suggestion offered above that there is some coördination in the growth of the peripheral fibres and the appear-

ance of the taste buds seems much more in accordance with the facts.

A good deal of interest attaches to the dorsal extension of the epibranchial ganglion. There is nothing in its composition or mode of development to indicate that it is formed otherwise than by the extension dorsally of the epibranchial placode. If, however, there are lateral mass cells in this ganglion they must enter into its composition at this point, and for this I find no evidence.

Herrick ('01) states that he finds no fibres derived from this ganglion except gustatory or special visceral, and since there is no evidence that cells other than those derived from the epibranchial placode enter into its composition we are driven to the conclusion that the epibranchial placode furnishes those cells which give rise to gustatory fibres and that those cells which give rise to general visceral fibres in the IXth ganglion of other species must come from the lateral mass or from the neural crest.

The evidence for the presence of general visceral fibres in the IXth nerve is exactly like the evidence for the presence of lateral mass cells in the ganglion. Neither has been detected. There is a possibility of both having been overlooked, of course, but if they should be found to be present it would not in my estimation affect the conclusion drawn. It would simply fall short of a demonstration. The fact that the VIIth, IXth and various portions of the Xth ganglia possess gustatory fibres approximately in proportion to the extent to which the epibranchial placodes contribute cells to these ganglia materially strengthens the conclusion. The geniculate ganglion of the VIIth has a well defined lateral mass contingent, as was shown in describing this ganglion. That portion of the visceral Xth from which arise the large visceral rami is almost exclusively derived from the lateral mass, containing only small contingents from the reduced fifth and sixth epibranchial placodes. The number of gustatory fibres derived from this ganglion is correspondingly small.

The third and fourth epibranchial ganglia possibly contain small constituents from the lateral mass. These ganglia, however,

are in such close proximity to the large lateral mass ganglion of the Xth that general visceral fibres might easily grow into the nerves, so that we cannot infer the presence of lateral mass cells simply because general visceral fibres may be in the peripheral nerves of these ganglia. The IXth is unique in that neither general visceral fibres nor lateral mass cells have been found in its composition.

Aside from the explanation given above, there seems to be no reason for the presence of the epibranchial placodes in the head, and for the part they play in contributing cells to those ganglia only which supply fibres to gustatory organs. The gustatory organs are all innervated from the cerebral ganglia, and only those cerebral ganglia give rise to gustatory fibres which contain cells derived from the epibranchial placodes, and the ganglion which seems to give raise to gustatory fibres only seems also to come only from the epibranchial placode.

My series at this stage are taken four hours apart, and the epibranchial ganglion acquires a connection with the proximal portion of the lateralis IXth (figs. 58 and 59) before losing its connection with the epidermis. However, the growing point of the epibranchial root is extremely thin. It passes up internal to the cardinal vein, which is in striking contrast with the growing parts of the epibranchial ganglia of the second and third true gills which pass up toward the brain external to the same vein (figs. 65, 66, 67, 68, and 69). The growing point of the IXth between the thicker portion derived from the epibranchial placode and the lateralis IXth, is shown in fig. 58. In the preceding series (75 hours) this connection is only one cell thick and in an embryo of 69 hours it is entirely absent.

THE ORIGIN OF THE LATERALIS IXth GANGLION

After reaching the level of the auditory vesicle in its dorsal extension, the growing point of the root of this epibranchial ganglion comes into contact with a mass of cells derived from the posterior and ventral portion of the auditory vesicle, which later forms the lateralis ganglion of the IXth. It is not derived from

nor directly related to the Xth, nor is it at first in contact with the auditory ganglion proper, but arises separately and can be followed continuously until it assumes the relations ascribed to it in the adult by Herrick ('01). At one stage in the enlargement of the auditory vesicle its relations are somewhat confused on account of the fact that the vesicle grows back and around the ganglion, but it can be followed, as mentioned, continuously. There is a later stage also where the lateralis IXth comes into contact with the motor root of the IXth and before the motor root has grown ventrally as far as the root of the epibranchial ganglion, when it is impossible to distinguish between the lateralis cells and the cells of the growing motor root. This condition is illustrated in fig. 59. In this stage we have the lateralis ganglion and the motor root closely combined. The constituents which can be positively identified as entering into the IXth ganglion are: first, the cells derived from the epibranchial placode situated in the distal portion of the ganglionic mass in the early stage, and extracranially in the latter stages: and secondly, the proximally situated lateralis ganglion, derived from the posterior portion of the auditory vesicle and closely associated with the motor root in its early stages, and in the later stages situated intracranially.

In discussing the differentiations of the postauditory lateral mass (p. 349–50) attention was called to the appearance in one series of a slight condensation of the lateral mass derivatives, in the region of the IXth ganglion just posterior to the auditory vesicle. This condensation appears in some series and not in others, so that one cannot be positive that no cells derived from the lateral mass enter into the proximal or lateralis portion of the IXth ganglion in its later stages. The early stages of the lateralis IXth are quite definite in origin and distinct in outline, and if lateral mass cells do enter into its composition they would be homologous to the preauditory lateral mass, and the ganglion would have a double composition and would resemble the VIIIth, if this ganglion contains lateral mass cells or the lateralis VIIth, rather than the lateralis Xth. The relations are difficult to unravel here, partly because of the presence of the motor root and partly because the cells

derived from the lateral mass are hard to distinguish from the surrounding mesectoderm. One can go no farther than to say that there is no well defined group of lateral mass cells entering into the lateralis IXth ganglion. The condition found in most of my series during the early proliferation of cells from the auditory vesicle before the definitive lateralis ganglion is formed, is illustrated in fig. 63. The lateralis IXth after it assumes a definite form is carried posteriorly by the backward growth of the auditory vesicle until it comes into the region of the slightly denser mass of mesectoderm, which at the time the lateralis IXth can first be detected is about seven sections posterior to the vesicle. This loose mass of cells, as mentioned above, is present in one of my series (A. nebulosus, Stage VII, figs. 40, 41,*G*), but in only one is it at all definite or does it present the appearance of an early stage of a ganglion. I take it to be the mass of cells which other authors have described as neural crest cells that enter into the IXth ganglion.

The early appearance of the definitive lateralis IXth ganglion is somewhat variable. It may not appear on both sides of the same embryo at the same time, and may vary in position, lying either on the ventro-mesial (fig. 61) or on the ventro-lateral side (fig. 62) of the posterior portion of the auditory vesicle. The definitive ganglionic mass may appear as a few loosely joined cells, some of which are still not entirely detached from the wall of the vesicle.

As in the case of the auditory ganglion, one can be quite sure that the bulk of the lateralis IXth ganglion is derived from the auditory vesicle. In the early stage of the vesicle there is no ganglionic mass in the position later occupied by the lateralis IXth, and the walls of the posterior end of the vesicle are clean cut and regular. Somewhat later the posterior wall becomes several cells thick, mitotic figures are numerous and a mass of cells is found attached to the ventro-mesial or ventro-lateral portion of the vesicle. No definite boundary can be determined for the forming ganglion, and cells are found in all positions between the wall of the vesicle and the body of the ganglion (fig. 63).

There is a well defined space, however, between the anterior end of the lateralis IXth and the posterior end of the auditory ganglion. The backward growth of the vesicle carries the ganglionic mass of the lateralis IX into the region occupied in some series by the condensation of the lateral mass, so that it is impossible to tell whether these lateral mass cells enter into the lateralis IXth or not. If they do, the lateralis IXth resembles the auditory rather than the lateralis Xth, which is derived solely from the postauditory placode.

Of the four acustico-lateralis ganglia in Ameiurus, the double ganglion belonging to the VIIth is derived solely from the lateral mass and shows its affinities with the general cutaneous Gasserian ganglion most closely, not only in the source from which it comes but in its mode of origin.

The lateralis Xth, derived from the postauditory placode, is decidedly unlike the lateralis VIIth, both in general appearance and mode of origin. It is from the early stages a well defined rod-like mass of cells whose long axis coincides with the long axis of the body, and is derived exclusively from the postauditory placode. Intermediate between these two extremes lie the auditory and lateralis IXth, which come largely if not exclusively from the auditory vesicle, but may have lateral mass cells in their composition.

The lateralis Xth is the most highly specialized of the four, and the reversal of the order of specialization, which usually proceeds from anterior to posterior, is at first sight striking. The whole acustico-lateralis system is, however, strictly a cranial specialization, the ganglia and nerves appearing first in the head and extending from there to the body, and having its center in the cranial region. The change from the generalized lateralis ganglion of the VIIth to the specialized lateralis ganglion of the Xth is in line with the order of appearance of the lateral line organs, which arise first in the cranial region, and later appear on the body. While usually a highly specialized structure lies anterior to a less specialized one, in the case of the acustico-lateralis ganglia the structure having a specialized mode of origin lies posterior to the one with a more generalized mode of origin.

THE ORIGIN OF THIRD AND FOURTH EPIBRANCHIAL PLACODES

The history of the first placode entering into the composition of the Xth ganglion, the third epibranchial, resembles very closely that of the epibranchial placode of the IXth ganglion, except that it forms its attachment to the brain in conjunction with the remaining roots of the Xth, by extending posteriorly until it joins the roots of the fourth placode. It can be detected first in my series (fig. 64) in a 69-hour embryo, at which stage it resembles very closely the early stage of the placode of the IXth nerve. In the next series (75 hours) the placode has proliferated quite a mass of cells mesially, but is not free from the epidermis at any point in its length, although three sections from its anterior end there is an evident constriction between the ganglionic mass and the epidermis.

In an 81-hour embryo the ganglion is not in contact with the epidermis at its posterior end, and has acquired a connection with the mass of cells lying over the site of the fourth and fifth epibranchial placodes of the Xth (fig. 79). In an 86-hour embryo the ganglion is still attached to the epidermis, but is somewhat larger and its root somewhat more evident. In a 93-hour embryo (figs. 80 and 65) the ganglion is still attached at its anterior end. In fig. 66, 93 hours, taken from a section following 65, the ganglion is attached by a very narrow neck of cells and throughout the remainder of its length is entirely free (figs. 67, 68, 69, 70) extending posteriorly into a narrow neck of cells which connects it with the last division of the Xth nerve. The root of this nerve, as mentioned above, lies lateral to the cardinal vein, as do all the other placodal roots of the Xth. The appearance of the epidermis near the placode resembles closely that in the region of the IXth. Anterior, dorsal and posterior to the placode the epidermis is usually one or two cells thick and quite ragged, presenting the appearance of proliferating cells into the mesectoderm. The thickening of the placode extends ventrally in the epidermis and, as in the case of the IXth, this region presents the appearance of furnishing most of the cells that move into the ganglion. Mitotic figures are occasional both in the epidermis and the placode.

There seems to be no question that here, just as in the case of the second placode, we have first a thickening of the epidermis above and behind the second true gill slit, followed by a proliferation of cells dorso-mesially to form the body and root of the ganglion, and later the complete detachment of the proliferated mass from the epidermis. The attachment disappears last at the extreme anterior end in an embryo of 99 hours. The dorsal extension of this placodal ganglion resembles closely the dorsal extension of the placodal ganglion of the IXth nerve; but the fact that it grows back parallel to the dorsal extension of the fourth epibranchial ganglion, and that both soon come into contact with the lateral mass ganglion of the Xth whose boundaries are at this stage not well defined and which is surrounded by mesectoderm make it more difficult than in the case of the IXth to determine whether there are lateral mass cells in its composition. General visceral fibres, if present in this ganglion, might be traceable either to lateral mass cells incorporated into the ganglion, or they might easily grow into it from the large lateral mass ganglion of the tenth, situated over the fourth and fifth gills. The conditions are by no means as favorable for drawing a definite conclusion as to the composition of the ganglion as in the case of the epibranchial ganglion of the IXth.

The development of the fourth epibranchial placode which gives rise to the fourth epibranchial ganglion resembles closely that of the second and third. In an embryo of 81 hours the posterior extension of the placodal ganglion has come into contact with the lateral mass ganglion situated just over the fourth gill slit at a point just ventral to that at which the root of the second epibranchial ganglion joins the same mass (fig. 79).

I have been unable to detect the placode in a 69-hour embryo. My 75-hour embryo is defective at this point. In the 81-hour embryo the ganglion is in contact with the epidermis throughout about half its length. The conditions are so similar here to those of the IXth, and first placode of the Xth, that I am sure that the method of appearance is the same. In fig. 68 (93 hours) is shown the appearance of the placode at the anterior portion of its attachment to the epidermis. Fig. 69 is taken two sections posterior

to 68 and shows the complete detachment of the posterior end of the ganglion from the epidermis. Fig. 70 shows the appearance of this ganglion seven sections posterior to the section from which fig. 69 was drawn, and just anterior to its union with the anterior portion of the lateral mass ganglion over the fourth gill slit. Fig. 71 shows the point of union of this ganglion with the anterior end of this lateral mass ganglion. The only difference between this placode and the third epibranchial lies in the thickness of its posterior portion, that part corresponding to the root of the third epibranchial ganglion. It lies much nearer its destination and apparently does not become so attenuated in reaching it. The later history of this ganglionic mass (figs. 81 and 82, *Ep. G. IV*) shows that it fuses much more closely with the ganglionic mass lying posterior to it than does the third, and may contain lateral mass cells, although I cannot be sure that they enter into its composition. It becomes completely detached from the epidermis in an embryo of 105 hours; while both the third and fourth epibranchial ganglia can be recognized in my oldest series, the fourth fuses much more closely with the remainder of the Xth than does the third.

THE ORIGIN OF THE FIFTH AND SIXTH EPIBRANCHIAL PLACODES

In the case of the fifth and sixth epibranchial placodes conditions are quite different on account of the fact that the lateral mass enters so prominently into the composition of this portion of the Xth. The relations are somewhat confused here on account of the enormous size of the lateral mass at the time the fifth and sixth placodes appear. The earliest trace of a placode I have been able to find for the fourth gill slit is represented in fig. 70 (93 hours). This placode has the appearance that all the other placodes present at the time they begin to proliferate cells mesially. The appearance usually presented in my preparations is shown in figs. 72 and 73. The large lateral mass ganglion comes into contact with the epidermis and remains in contact about the length of time the other placodes retain their connection with the epidermis. Except for the condition shown in fig. 70 it would not be possible to as-

sert that we had a true placode here. However, I believe that the contact of the lateral mass ganglion with the epidermis is purely a secondary matter and that while it cannot be proven, in all probability the fifth epibranchial placode is contributing cells to the lateral mass ganglion. I can see no other interpretation for the presence of the early stage of the placode before the contact, or the persistance of the contact during the time usually occupied in the proliferation of cells from the placode. As mentioned above, however, I have been unable to separate this ganglionic mass into lateral mass and placodal portions.

Fig. 72 is taken through the middle of the fifth epibranchial placode of a 99-hour embryo. The attachment of the lateral mass to the placode is here slightly anterior to the anterior end of the lateralis Xth, which does not appear in the figures. Fig. 73 is from an embryo of 105 hours. The lateralis Xth and lateral mass are cut through the anterior portions. The placodal thickening is seen to extend ventrally in the epidermis as do those of the second, third and fourth placodes. The attachment to the epidermis does not continue up to the 113-hour stage, and judging by the distance of the ganglion from the epidermis in this stage may disappear some time before this, perhaps between the 105 and 113-hour stages.

The sixth epibranchial placode is less prominent than the fifth. The lateral mass ganglion retains its contact a shorter time with the epidermis, which is slightly thickened, and I have not been able with certainty to identify the placode previous to the time the contact is formed. The series from which I describe it are taken from 6 to 8 hours apart, and this is not sufficiently close to be sure that the placode is not visible before the time of contact of the lateral mass with the ectoderm.

In an embryo of 113 hours there is present a second contact between the lateral mass cells and the epidermis. This contact occurs at the posterior end of the lateral mass portion of the Xth ganglion, which is quite large at this time and extends back of the fifth gill slit. The contact is present in an embryo of 113 hours from which fig. 74 was drawn, and must have been formed earlier but does not continue as long as the 120-hour stage. I have been

unable to determine to what extent cells move from the placode into the ganglion, since there seems to be no noticeable distinction between placodal cells and lateral mass cells in any of my embryos. The same reasoning applies here, however, that was used in the case of the fifth epibranchial placode. The location, time of appearance, and manner of thickening of the epidermis resemble the third and fourth placodes in their early stages when they are undoubtedly contributing cells to these ganglia. Its more transient character is in keeping with the reduction which has occurred in the fifth epibranchial ganglion as compared with the fourth, but seems more marked. It indicates a reduction of the placodes from posterior to anterior, and is to be associated with the reduction in the number of gills in the bony fishes as compared with cyclostomes and elasmobranchs.

The time at which the contact occurs and the length of the attachment of the IXth and the four epibranchial ganglia of the Xth is shown in the following table:

TABLE IV

Showing the time at which the epibranchial placodes of the ninth and tenth ganglia appear, the time at which they become detached, and the length of time of attachment.

EPIBRANCHIAL PLACODES	TIME OF FIRST APPEARANCE	TIME OF DETACHMENT	LENGTH OF TIME OF ATTACHMENT
	hours	*hours*	*hours*
Second	56½	93	37½
Third	69	99	30
Fourth	75	105	30
Fifth	93	−113	−20
Sixth	105+	113+	+−8

This table shows that the fifth and sixth epibranchial placodes appear in serial order and become detached from the epidermis in the same order. They differ principally in having a shorter total time of attachment. There seems to be no reason for supposing that conditions are different here other than in the reduction of the placodes and in the presence of a well defined lateral mass ganglion which fuses with the placode.

The condition here seems to throw some light on the interpretation given these placodes by former workers. They have so often been described as arising at the time the neural crest ganglion fuses with the epidermis that I am unable to reconcile the conditions existing, under which the placodal ganglia of the IXth and first two divisions of the Xth arise in Ameiurus, with these descriptions, unless there are neural crest cells in the IXth and first two divisions of the Xth, which fuse with the epidermis in an early stage in these types. Until the nerve components have been thoroughly worked out in these types in which the neural crest ganglia have been described as entering into the IXth and first two anterior divisions of the Xth, so that we can be sure as to whether there are general visceral fibres in these nerves, it is useless to speculate as to either their mode of origin or composition. The presence of a neural crest in the region of the IXth and Xth nerves, however, does not prove the presence of neural crest cells in these ganglia, unless they can be definitely traced into them. The absence of a lateralis ganglion and fibres in the IXth nerve in Menidia (Herrick, '99) and apparently in Gadus (Herrick, '00, p. 296) and its presence in Ameiurus (Herrick, '01) indicate how useless it is to speculate as to the composition of these ganglia in one type because we know them in another which is closely related.

LATER HISTORY OF THE LATERAL MASS GANGLIA OF THE Xth

There are two of these ganglia in Ameiurus: (1) the small general cutaneous ganglion, the jugular, situated intra-cranially (Herrick, '01, p. 210). This ganglion is present in Gadus (Herrick, '00, p. 297), where it is also intra-cranial. In Menidia, however, the ganglion is small and wedged in between the visceral ganglia and is extra-cranial.

(2) Beside the jugular ganglion there is the large visceral ganglion situated extra-cranially over the fourth and fifth gill slits, the earlier history of which was taken up with the postauditory lateral mass. Some idea of its size at the time it comes into contact with the placodes of the fourth and fifth gill arches can be obtained from

fig. 73 which, however, is not taken through the largest portion of the ganglion. Herrick ('01) does not give a description of the communis ganglion of the Xth nerve in Ameiurus further than to call attention to the fact that it is typical, and in describing Gadus ('00) calls attention to the fact that it is similar to Menidia. In Menidia (Herrick, '99) the fifth branchial nerve is larger than the other four and gives rise not only to the fibres for the fifth gill arch but also to the fibres for the great visceral and œsophageal rami of the vagus. He also calls attention to the fact that in Menidia the ganglia of the glossopharyngeus and first branchial ganglia of the vagus are composed of very large cells with medium and small cells intermingled among them, and that as we go toward the caudal end of the ganglionic complex, while there are still found cells of various sizes, the smaller ones become increasingly numerous, and suggests the hypothesis that the larger cells are related to taste buds and the smaller ones to visceral fibres.

The division of the extra-cranial Xth ganglion into four parts can hardly be so distinct in Ameiurus as Herrick describes it in Menidia, since in my oldest series the last two divisions are not easy to distinguish and the fusion is probably much more marked in the adult. At the time the fifth and sixth placodes of the Xth nerve are present the lateral mass ganglion appears as a dense mass of cells lying over the fourth and fifth gill slits. The first two divisions of the Xth which are placodal in origin can be distinguished easily, and the first of these in any series is distinct up to a late stage.

We have here in the Xth nerve, then, a general cutaneous or jugular ganglion derived from the lateral mass and lying intracranially, and an extra-cranial visceral ganglion in which it is not possible to separate the placodal cells from the lateral mass cells which greatly predominate over the cells derived from the fifth and sixth epibranchial placodes. This whole ganglionic mass is described by Herrick as a communis ganglion. We must add to this large extra-cranial mass the two anterior placodal ganglia which however can be distinguished easily from the combined lateral mass and placodal portion. These relations are rendered clear by figs. 79, 80, 81, and 82.

The embryological evidence based on the mode of origin of the Xth ganglion strongly supports Herrick's suggestion, based on a study of the adult condition in Menidia, since the general and special visceral ganglionic cells come from two different sources although combined into one ganglionic mass.

The intra-cranial or jugular ganglion is the last of the cranial ganglia to assume definite form, and can be recognized first as a definitive ganglionic mass in an embryo of 113 hours. At this time the backward growth of the ear has carried the lateralis IXth quite near to the future jugular ganglion, but they are separated here as in all preceding stages by a blood vessel. Between the 69-hour stage and the 113-hour stage the jugular ganglion is not definitely formed and appears gradually as a mass of cells surrounding the root of the Xth nerve and extending from near the medulla down around the root of the nerve to the extra-cranial portion. Preceding the stage of 69 hours, and particularly before the formation of a fibrillated root, it is not possible to distinguish a definitive jugular ganglion from those cells which will form the extra-cranial portion. The history of the jugular ganglion seems to be briefly as follows: First there is a loose mass of cells extending from the brain ventrally to a point where the epibranchial placodes will be formed (fig. 75). This is followed by a stage in which the visceral ganglion develops and the fibrillated root is present, and in which the jugular ganglion surrounds the root, sometimes thicker on one side, sometimes on the other, and extends down over the root to the enlarged extra-cranial portion. This is followed by a stage in which there is a definitive ganglionic mass present, which owing to the development of the cartilaginous skeleton can be designated as intra-cranial. This is connected with the extra-cranial portion by a mass of spindle shaped cells only, lying on and among the fibres of the root of the Xth nerve. These spindle shaped cells I interpret as sheath cells, so that the jugular ganglion is now quite distinct and can be easily differentiated from the extra-cranial communis ganglion after 113 hours in Ameiurus.

There is little change in the appearance of this ganglion until the stage of 81 hours, when the fibrous root of the Xth nerve is

present. The ganglion cells then surround the proximal part of the root as indicated in fig. 76, which is taken through the middle of the root and is not exactly transverse to the long axis of the embryo, so that nearly the whole length of the fibrous root shows. The cells occupying the proximal portion of the root where the definitive jugular ganglion appears surround the root, lying anterior and posterior to it.

There is little difference in size between the cells which occupy the position in which the ganglion is later found and those cells surrounding the future root. This condition persists for some time, but in an embryo of 113 hours there is a decided increase in the number and a change in the appearance of the cells as shown in fig. 77, which is taken through the middle of the root. Anterior and posterior to this point the cells are usually grouped on either side of the fibrous portion of the root.

A much later stage (fig. 78) from an embryo of 138 hours shows this ganglion after it has become a prominent portion of the vagus complex. The manner in which the ganglion cells are distributed through the root and their small size explains the difficulty one finds in separating the cells of the future ganglion from the spindle shaped cells of the root. The extra-cranial portion of the lateral mass I shall not describe further in detail. It is so large and increases in size so rapidly that it is by far the most conspicuous structure in this region of the body. It is too large, in fact, to draw under a camera at the same magnification I have used for the other portions of the ganglia. The figures (72 and 73) show it in the early stages and figs. 79, 80, 81 and 82 show its relative, size and relations. There is never any difficulty in locating the bulk of this ganglion after the 81st hour. There is the difficulty of determining its dorsal boundary, mentioned above, since it extends as a thin mass of cells dorsally to join the jugular. Its separation from the jugular is in all but these early stages, quite distinct.

The fact that this lateral mass ganglion consists of two parts, an intra-cranial which is general cutaneous, and an extra-cranial which is visceral supplying visceral surfaces, has an interesting bearing on the relation of spinal and cranial nerves.

The spinal ganglia derived from the neural crest furnish both general visceral and general cutaneous fibres, but these two components are combined in the same ganglion, although probably having separate terminations in the cord. In the head, however, we have the lateral mass ganglion of the Xth, which probably corresponds to the neural crest ganglion of other types, differentiated into two ganglia, a general cutaneous, the jugular, situated intracranially, and an extra-cranial portion that is in all probability the general vesceral ganglion.

From the preceding description it will be seen that there are two quite distinct sources of origin for the cerebral ganglia in Ameiurus, the lateral mass and the epibranchial placodes. Unlike most types, there is no well defined neural crest. The lateral mass early gives rise to the dorso-lateral placodes represented by the auditory vesicle and the pre- and postauditory placodes, while the remainder of the lateral mass gives rise to the primordia of ganglia and to mesectoderm. The lateral mass doubtless contains beside the dorso-lateral placodes the homologue of the neural crest of other types, but the greater portion goes to form mesectoderm.

Of the components found in the adult ganglia, the special visceral or gustatory come from the epibranchial placodes, while the general visceral come from the ventral portion of the lateral mass. The general cutaneous component comes from the lateral mass also, but typically from the dorsal portion, as in the case of the jugular ganglion. The acustico-lateralis component shows its kinship to the general cutaneous component in that the lateralis ganglia of the VIIth come from the lateral mass. The auditory and lateralis IXth come chiefly from the auditory vesicle but may have lateral mass cells in their composition. They show a somewhat more specialized mode of origin, while the lateralis Xth comes entirely from the postauditory placode and is the most highly specialized in mode of origin of the acustico-lateralis ganglia. The geniculate ganglion was seen to be composed of constituents from both the ventral portion of the lateral mass and from the epibranchial placodes.

The visceral ganglion of the IXth nerve seems to be a pure placodal ganglion. The first two epibranchial ganglia of the Xth resemble the IXth, but may possibly contain lateral mass cells. The last two epibranchial ganglia of the Xth are quite small and combine with the large lateral mass portion so that the posterior portion of the visceral Xth is largely lateral mass in origin. These relations are shown in table V:

TABLE V

Showing the source of the various components of the cranial ganglia of Ameiurus. The presence of any given component is indicated by the word present.

DERIVATION		V	VII	VIII	IX	X	X	X	X	COMPONENT
Lateral Mass	Dorsal portion	Present						Present	Present	General cutaneous
			Present	?	?					Acustico-lateralis
	Dorso-lateral placodes			Present	Present			Present	Present	Acustico-lateralis
	Ventral portion		Present			?	?	Present	Present	General visceral
Epi-branchial placodes			Present		Present	Present	Present	Present	Present	Special visceral

In this table the general cutaneous, general visceral and acustico-lateralis portions of the Xth are placed over the last two epibranchial ganglia not so much to indicate their segmental position as because they occupy this relative position. The lateralis Xth extends however much posterior to the visceral portion.

A comparison of this table with table II compiled from Herrick's work on Ameiurus (p. 321) shows that the ganglia are as discrete in their mode of origin as are the components of the adult ganglia

and nerves, with the single exception that we have portions of the acustico-lateralis ganglia, as shown in other types, arising from the same source as the general cutaneous. This is to be explained on the basis of the relationship of the two components. In sharp contrast with this, however, we have distinct sources of origin for the special and the general visceral ganglia which are combined in the preceding table (II) and which in the adult are closely fused, particularly in the geniculate and in the posterior portion of the tenth.

The differences between the two tables may be summarized briefly as follows: The visceral ganglia of the adult in table II (p. 321) are broken down in the table V into those portions derived from the epibranchial placodes, *i.e.*, the special visceral or gustatory ganglia, and the portions derived from the lateral mass, *i.e.*, the general visceral.

The acoustico-lateralis ganglia of the adult in table II are broken down in table V into those portions derived from the lateral mass, *i.e.*, the lateralis VIIth and possibly portions of the VIIIth ganglion and of the lateralis IXth, and those portions derived secondarily from the auditory vesicle and placodes, *i.e.*, all of the lateralis Xth and most if not all of the auditory and lateralis IXth ganglia.

GENERAL SUMMARY

1. The neural plate in Ameiurus differentiates longitudinally into three regions: a median region, the neural keel, which later becomes the neural tube, and two lateral regions, the lateral masses, separated from the neural keel by constricted areas.

2. After the body has assumed a rounded form, the lateral masses come to lie on the sides of the body still retaining their connection with the neural cord by constricted areas. Part of the lateral mass on either side differentiates into the auditory vesicle and the pre- and postauditory placodes, which are extensions of the vesicle and resemble it in structure. These represent the dorso-lateral placodes of other authors. The remainder of the lateral mass breaks down more or less completely into loose tissue in

which the general cutaneous, general visceral and some of the acustico-lateralis ganglia form, the remainder not thus used going to form mesectoderm. In the auditory region all of the lateral mass is converted into the auditory vesicle.

3. The Gasserian ganglion arises near the anterior end of the lateral mass, over the mandibular bar, and is first recognizable as a slightly denser area on either side of which the lateral mass breaks down into mesectoderm. Just posterior to this region the ventral portion of the lateral mass, over the hyoid bar, gives rise to part of the geniculate ganglion which later combines with the portion derived from the epibranchial placode, the two constituents not being separable shortly after fusion.

4. Just anterior to the auditory vesicle a portion of the lateral mass gives rise to the lateralis VIIth ganglia which does not differentiate into the dorso-lateral and ventro-mesial ganglia until later. The posterior end of this ganglionic mass is in contact with the auditory vesicle. Both the Gasserian and lateralis VIIth, and that portion of the geniculate derived from the lateral mass are, at first, small and ill defined, with irregular borders which pass almost imperceptibly into mesectoderm. Between the regions in which these ganglia appear the lateral mass breaks down into mesectoderm.

5. The auditory ganglion arises chiefly, if not exclusively, by the proliferation of cells from the anterior end of the auditory vesicle, but in such close contact with the preauditory lateral mass that one cannot be certain that there are no lateral mass cells in it.

6. The lateralis ganglion of the IXth nerve arises also chiefly, if not entirely, by proliferation of cells from the posterior end of the auditory vesicle, but it is carried by the backward growth of the vesicle into the region of the root of the IXth nerve, where a slight condensation of lateral mass cells is sometimes present, and it may possibly contain lateral mass cells.

7. Between the IXth and Xth ganglia and for some distance posterior to the anterior end of the Xth, the lateral mass breaks down completely into mesectoderm. In the region of the Xth nerve the dorsal portion of the lateral mass breaks down into

loose tissue, in which the general cutaneous jugular ganglion later appears, while the ventral region which forms the general visceral portion of the Xth retains its continuity to a greater extent. The ventral region of the lateral mass which enters into the Xth ganglion resembles in appearance and corresponds in position to that portion of the preauditory lateral mass which enters into the geniculate ganglion.

8. The preauditory placode which is the anterior continuation of the auditory vesicle extends as far forward as the hyoid gill slit, but between the ear and the hyoid gill slit it breaks down completely into mesectoderm. Its anterior extension disappears at the exact point where the epibranchial placode of the hyoid arch appears but seems to have no direct relation to it other than in its position. The preauditory placode does not give rise to the preauditory lateral line organs, there being a period of some hours between the disappearance of the placode and the appearance of the first lateral line organs.

9. The postauditory placode, which is the posterior extension of the auditory vesicle, becomes detached from the vesicle and moves back by successive differentiations of the epidermis to a point back of the fourth lateral line organ, where it gradually disappears. Before its disappearance there have appeared four lateral line organs anterior to it and at least two posterior to it. It disappears in the region of the fifth, but probably does not give rise even to that. The lateralis Xth ganglion arises by the proliferation of cells from the postauditory placode after it has moved some distance back of the auditory vesicle. After the placode ceases to contribute cells to the ganglion it moves beyond the posterior limit of the ganglion, losing all connection with it, and does not give rise to the lateral line nerve.

10. Both the pre- and postauditory lateral line organs are formed by gradual differentiations of the deeper layer of the epidermis, sometimes singly, sometimes two from a common primordium and are entirely distinct in origin from the placodes.

11. The epibranchial ganglia all have a common mode of origin. The epibranchial placode of the hyoid arch appears first as a thickening of the epidermis dorsal and slightly posterior

to the point of contact of the endodermal pocket of the hyoid gill slit with the epidermis. This thickening is characterized by the irregular arrangement of its nuclei and by the large number of mitotic figures. The thickening of the epidermis is followed by an active proliferation of cells mesially, which come into contact with the ventral portion of the lateral mass in this region. The proliferated mass later becomes detached and after some hours the geniculate ganglion, which is thus composed of a lateral mass contingent and a placodal contingent, assumes definite form and comes into intimate relation with the Gasserian ganglion.

12. The epibranchial placode of the first true gill slit arises in a similar manner, appearing first as a slight thickening lying dorsal and posterior to the first gill slit. The thickening is accompanied by active mitosis, proliferation of cells, and finally by complete detachment, *en masse*, of the proliferated cells. The proliferated epibranchial ganglion is in this case, however, apparently a pure placodal ganglion, since no lateral mass cells could be detected entering into its composition. Its dorsal extension comes into contact with the remainder of the IXth before the ganglion is completely detached from the epidermis however.

13. The epibranchial ganglia of the second and third gill arches have exactly similar modes of origin, but their dorsal extensions soon come into contact with the lateral mass portion of the Xth. While the second and third epibranchial ganglia are definite in outline and mode of origin, their proximity to the Xth makes it difficult to be sure that there may not be lateral mass cells in their composition.

14. The epibranchial ganglion of the fourth and fifth true gills, owing to the fact that they are in the region of the large lateral mass ganglion of the Xth, present a somewhat different history. The fourth can be detected before the lateral mass of the Xth comes into contact with it, and while its early stages resemble those anterior to it, it does not become detached before the fusion occurs between it and the lateral mass portion. In the case of the fifth, the epibranchial ganglion cannot be detected in my series before the fusion, so that while there is every reason for thinking that the fifth and sixth epibranchial placodes contribute cells

to the Xth, the relative amount cannot be determined and the two components cannot be separated. The visceral ganglia of the second and third gill arches are apparently like the IXth, pure placodal ganglia with possibly a small contingent of lateral mass cells, while the remainder of the tenth is composed of a few placodal cells derived from the small fifth and sixth epibranchial placodes united to a large lateral mass contingent.

15. The ganglia described in this paper as general cutaneous, acustico-lateralis, and visceral, have been followed to a late stage and shown to be the ganglia described by the authors under these names. The evidence from Ameiurus is little short of a demonstration that there are separate origins for the general visceral and for the special visceral systems, the former coming from the ventral portion of the lateral mass, or, in other types, from the neural crest, and the latter from the epibranchial placodes. For the acustico-lateralis system, there are two sources of origin. The lateralis VIIth comes entirely from the lateral mass and in other types apparently from the neural crest. The auditory and the lateralis IXth come chiefly from the auditory vesicle but may contain lateral mass cells, while the lateralis Xth comes entirely from the postauditory placode. The description of the acustico-lateralis system as a special cutaneous system finds a strong support in embryological evidence. The lateralis VIIth ganglia represent an intermediate stage between the general cutaneous ganglia, and the acustico-lateralis ganglia of the IXth and Xth nerves, resembling the former in mode of origin and the latter in structure and function. The latter are derived from the dorso-lateral placodes represented by the auditory vesicle and postauditory placodes. The general cutaneous ganglia of the Vth and Xth come from the dorsal portion of the lateral mass also, or neural crest of other types.

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ABBREVIATIONS

Au. G., Auditory ganglion.

Au. G., I and II. First and second divisions of the auditory ganglion.

Au. Ves., Auditory vesicle.

Br. I, II, III, IV, V, First to fifth branchial nerves.

En., Endoderm.

D. L. VII, Dorso-lateral portion of the lateralis VII ganglion.

D. L. M., Dorso-lateral mass.

Ep., Epidermis.

Ep. Pl. I, First epibranchial placode (epibranchial placode of the **VII** nerve).

Ep. Pl. II, Second epibranchial placode (epibranchial placode of the **IX** nerve).

Ep. Pl. III, Third epibranchial placode (first epibranchial placode of the **X** nerve).

Ep. Pl. IV, Fourth epibranchial placode (second epibranchial placode of the X nerve).

Ep. Pl. V, Fifth epibranchial placode (third epibranchial placode of the X nerve).

Ep. Pl. VI, Sixth epibranchial placode (fourth epibranchial placode of the X nerve).

Ep. G. II, Second epibranchial ganglion (epibranchial ganglion of the IX nerve).

Ep. G. III, Third epibranchial ganglion (first epibranchial ganglion of the X nerve).

Ep. G. IV, Fourth epibranchial ganglion (second epibranchial ganglion of the X nerve).

G., Remnant of the lateral mass in the region of the lateralis IX ganglion.

Gass. G., Gasserian ganglion.

Gen. Com. X., General communis or visceral ganglion of the X nerve.

Gen. G., Geniculate ganglion.

J. G. X, Jugular or general cutaneous ganglion of the X nerve.

L. IX, Lateralis ganglion of the IX nerve.

L. G. X, Lateralis ganglion of the X nerve.

L. L. X, Lateral line nerve trunk of the lateralis X ganglion.

L. M., Lateral mass.

L. M. G. VII, Lateral mass ganglion (general visceral ganglion) of the geniculate.

Med., Medulla oblongata.

Mes., Mesoderm.

Mesec., Mesectoderm.

N. C., Neural canal.

Post Pl., Postauditory placode.

Pre. Pl., Preauditory placode.

R. X, Root of the X nerve.

R. Au., Root of the auditory nerve.

R. C. D. X., Ramus cutaneus dorsalis vagi.

R. D. L., VII, Root of the dorso-lateral portion of the lateralis VII ganglion.

R. Gass., Root of the Gasserian ganglion.

R. Gen., Root of the geniculate ganglion.

R. L. G. X, Lateralis root of the X nerve.

R. S. J, Ramus supra-temporalis J, glossopharyngei, (Herrick, '01).

R. V. L., VII, Root of the ventrolateral portion of the lateralis VII ganglion.

R. A. A., Ramulus aeusticus ampulæ anterioris.

R. A. E., Ramulus acusticus ampullæ extrenæ.

R. L., Ramulus acusticus lagenæ.

R. N., Ramulus acusticus neglectus.

R. P., Ramulus acusticus ampullæ posterioris.

R. Sac., Ramulus acusticus sacculi.

R. N., Ramulus acusticus recessus utriculi.

T. Gass., Trunk of the Gasserian ganglion (supero-lateral strand of Wright).

T. Gen., Trunk of the geniculate ganganglion of the VII nerve (infero-mesial strand of Wright).

T. V. L. VII, Trunk of the ventrolateral portion of the lateralis VII ganglion (hyomandibular nerve).

V. L. VII, Ventrolateral portion of the lateralis VII ganglion.

X, Mass of cells of unknown origin and whose fate is unknown, lying just anterior to the first epibranchial placode.

V. L. VII, Ventrolateral portion of the lateralis VII ganglion.

X, Mass of cells of unknown origin and whose fate is unknown, lying just anterior to the first epibranchial placode.

Y, Ventral portion of the lateral mass lying in the region of the IX and X ganglia.

EXPLANATION OF FIGURES

All sections are cut 7 micra in thickness, and all figures and reconstructions are taken from transverse sections. Figures were drawn with a camera lucida with objective 4 mm. eye-piece, × 8 Spencer, reduction to $\frac{1}{2}$. Total magnification, 186 dia.

1 (A. neb., Stage I) is taken at a stage when the medullary plate is slightly differentiated into the neural cord (N. C.) and the lateral mass (L. M.). The section lies just posterior to the point of formation of the optic cup.

2 and 3 (A. neb.) are taken from the same embryo, Stage II. Fig. 2 is taken six sections posterior to the optic vesicle and lies in a region just anterior to the point at which the Gasserian ganglion will form later. The lateral mass here breaks down completely into mesectoderm.

3 is taken just posterior to the future position of the Gasserian ganglion, twenty-nine sections posterior to the optic vesicle. The greater portion of the lateral mass here breaks down into mesectoderm also.

4 to 9 (A. neb., Stage III) are all from the same embryo which is slightly older than that from which figs. 2 and 3 were taken. Fig. 4 lies four sections anterior to the auditory vesicle and shows the differentiation of the lateral mass into the preauditory placode and the dorsal portion of the lateral mass which later forms the lateralis VII ganglion.

5 shows the differentiation of the lateral mass into dorso-lateral mass and post-auditory placode, four sections posterior to the auditory vesicle.

6 is taken through the middle of the auditory vesicle before the appearance of a definite cavity.

7 lies four sections posterior to the optic vesicle. It is taken through the same relative position as fig. 2, Stage I, and shows the transition of lateral mass into mesectoderm.

8 is taken through the region in which the Gasserian ganglion forms. The lateral mass does not break down into a loose mass of cells so early here as it does just anterior and posterior to the Gasserian ganglion.

9 lies five sections anterior to fig. 4 and nine sections anterior to auditory vesicle. It shows the reduction in size of the preauditory placode as one reads forward from the position of fig. 4.

2.
L M.
N C
3.
L M
N C
5.
N C
Post. Pl.
D L M.
1.
N C
L M.
8.
N C
L M
Mesec
6.
Au Ves
N C.
7.
Mesec
N C
Ep
4.
D L M
Pre Pl
N C
Mesec
9.
D. L.M
Pre. Pl
Mesec

EXPLANATION OF FIGURES

10 and 11 (A. neb.. Stage V) are from the same embryo. Fig. 10 is taken through the posterior end of the Gasserian ganglion at one of the earliest stages at which it can be definitely located. This section passes through the hyoid gill pocket (En.).

11 is taken through the posterior end of the lateralis VII ganglion and lies four sections anterior to the auditory vesicle.

12 (A. neb., Stage VII) is from an older embryo than that from which fig. 6 was taken. It lies in the mid-region of the vesicle and shows the absence of a lateral mass after the vesicle is formed.

13 is taken from the same embryo as that from which figs. 10 and 11 were taken (A. neb., Stage V). It lies four sections back of the anterior end of the auditory vesicle. The proliferation of the capsule cells to form the auditory ganglion is taking place here.

14 and 15 (A. neb., Stage IV) are from embryos of the same age. Fig. 14 lies two sections anterior to the anterior end of the auditory vesicle. The preauditory placode is here continuous with the vesicle; on the opposite side of the same embryo there is one section, fig. 15, intervening between the preauditory placode and the vesicle.

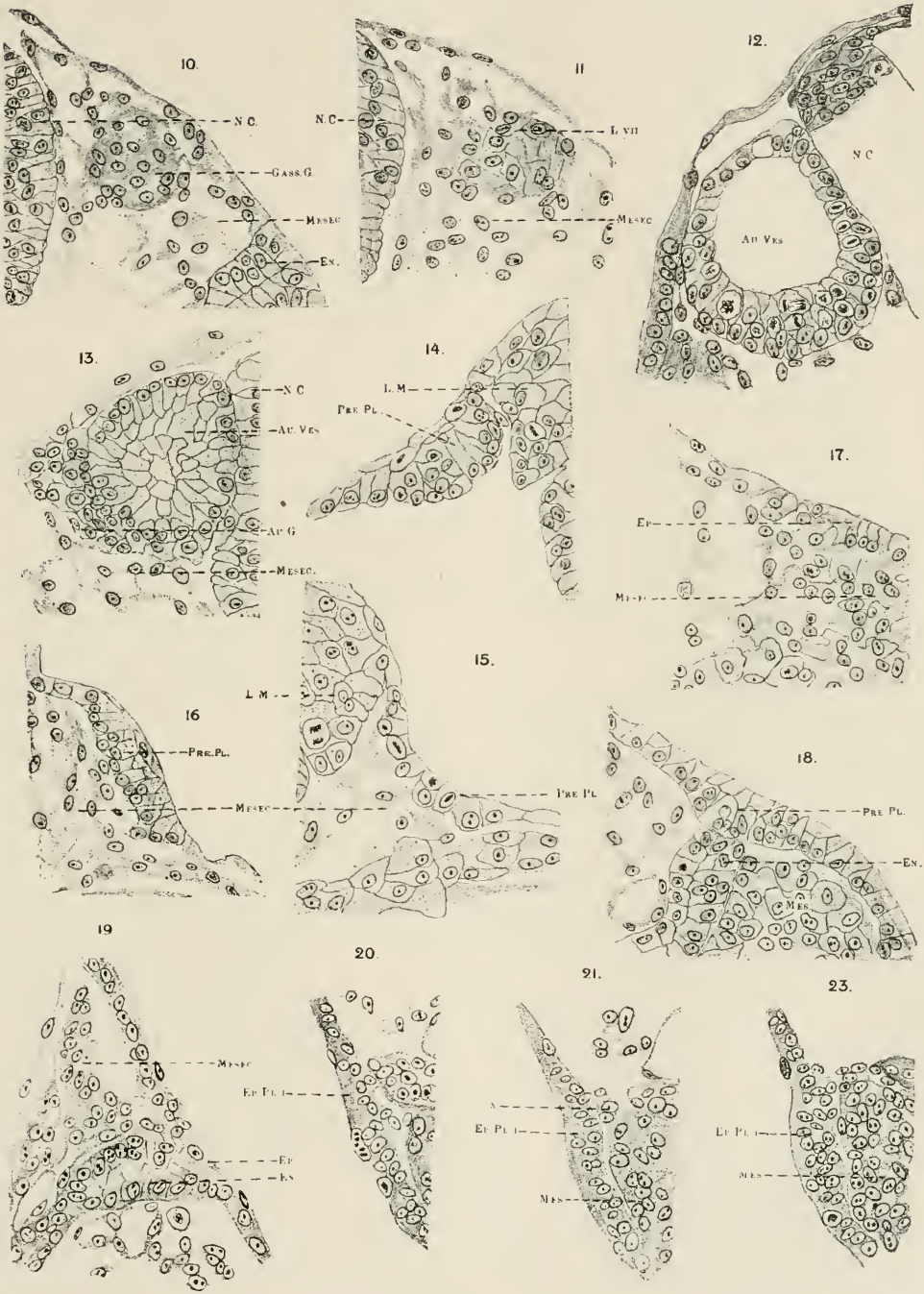
16 (A. neb., Stage IV) is from a slightly more developed embryo of the same age as figs. 14 and 15 and is taken through the middle of the preauditory placode. There are six sections in which the placode is absent between the posterior end of the placode and the anterior end of the vesicle.

17 (A. neb., Stage V) is taken from an older embryo than the one from which fig. 16 is taken and lies nineteen sections anterior to the vesicle. The placode is here disintegrating and is entirely absent in the remaining eighteen sections. It is intact anterior to this point.

18 from the same embryo as fig. 17 shows the appearance of the preauditory placode at the posterior third of its contact with the hyoid gill pocket which extends over eleven sections. This is the last recognizable stage of the placode. It still retains a slight resemblance to the earlier stages.

19 to 24 (A. neb., Stage VI) are all from the same embryo. Fig. 19 is taken through the mid region of the hyoid gill pocket and shows the complete disappearance of the preauditory placode.

20, 21, 22 and 23 are consecutive sections, fig. 20 lying at the extreme posterior end of the point of contact of the hyoid pocket whth the epidermis. The small mass of cells (X) lies anterior to the position of the future epibranchial ganglion and does not seem to enter into its composition. These sections show the gradual thickening and irregular arrangement of the cells in the epidermis (epibranchial placode) which precedes the proliferation of cells mesially.


10.
N.C.
GASS.G.
MESEC
EN.
11
N.C.
L. VII
MESEC
12.
N.C.
AU. VES.
13.
N.C.
AU. VES.
AU. G.
MESEC.
14.
L.M.
PRE. PL.
15.
L.M.
PRE. PL.
16
PRE. PL.
MESEC
17.
EP.
MESEC
18.
PRE. PL.
EN.
MES.
19
MESEC
EP. PL.
EP.
EN.
20.
21.
N.
EP. PL.
MES.
23.
EP. PL.
MES.

EXPLANATION OF FIGURES

24 lies four sections back of fig. 23, the epidermis is reduced in thickness and the placode is not so long dorso-ventrally. Just back of this the epidermis is of normal thickness.

25 to 30 (A. neb., Stage VII), illustrating the origin of the epibranchial portion of the geniculate ganglon, are all from the same embryo which is slightly older than the one from which figs. 19 to 24 were taken. Figs. 25 to 28 are consecutive. Figs. 29 and 30 are consecutive, one section intervenes between figs. 28 and 29. Fig. 25 lies near the posterior limit of the contact of the hyoid pocket with the epidermis and fig. 26 at the extreme posterior limit. Active cell division is taking place here. In fig. 27 the ganglionic mass is proliferated mesially over the hyoid pocket which is no longer in contact with the epidermis. In fig. 28 the ganglionic mass is still purely placodal in origin, but in fig. 29 the placodal ganglion is in contact with a slightly delimited mass (L. M. G. VII) derived from the lateral mass. In fig. 30 the lateral mass portion of the ganglion predominates. A few sections posterior to this point the ganglion is entirely of lateral mass origin and the epidermis is of normal thickness.

31 to 39 (A. melas, 86 hours) illustrate the relations of the Gasserian ganglion to the geniculate and the dorso-lateral and ventro-lateral lateralis ganglia of the VII nerve. Figs. 37, 38 and 39 also show this relation to the auditory ganglion and vesicle. Preceding the stage of 86 hours it is not possible to differentiate between the two lateralis ganglia of the VII nerve and following this stage the ganglia soon becomes condensed and it is difficult to unravel them.

31 is taken throughthe trunk of the nerve (supero-lateral strand of Wright) of the Gasserian ganglion.

32 is taken just anterior to the point of origin of the nerve (infero-mesial strand of Wright) of the geniculate ganglion. These two strands combine in a later stage some distance from the ganglia and then split into the maxillary and mandibular nerves.

33 is taken through the root of the Gasserian ganglion.

22.
X
Ep Pl I
Mes
25.
Ep Pl I
En
26.
Mes c
Ep Pl I
En
Mes
29.
Ep.
L M G VII
Ep Pl I
Mes.
30.
L M G VII
Ep Pl I
28.
Ep Pl I
Mes
En
27.
Mes c
Ep Pl I
En
Mes
31.
Ep
Gass G
I Gass
32.
Gass G
Gen G
Ep Pl I
24.
33.
R Gass
Gass G
Gen G

EXPLANATION OF FIGURES

34 is taken through the anterior end of the dorso-lateral lateralis VII ganglion and the root of the Gasserian.

35 is taken through the trunk (hyomandibular nerve) of the ventral lateralis ganglion of the VII and the root of the geniculate ganglion.

36 is taken through the root of the ventral lateralis ganglion.

37 is taken through the extreme anterior tip of the auditory ganglion. The dorsal lateralis VII runs through figs. 34 to 37.

38 and 39 are taken through the anterior and median portions of the auditory ganglion. The roots of the dorso-lateral, ventro-lateral and geniculate ganglia appear in figs. 37, 38 and 39.

40 to 43 (A. neb., Stage VII) are taken from the same embryo as figs. 25 to 30. Figs. 40 and 41 are consecutive sections and show the only recognizable trace of a condensation of the cells derived from the lateral mass in the region of the IX nerve, except the ventral portion of the lateral mass (**Y**) which does not enter into the IX ganglion.

40 is taken one section posterior to the auditory vesicle.

41 is taken two sections posterior to the auditory vesicle.

34
R. GASS.
D. L. VII
GEN. G
35.
D. L. VII
R. GEN
R. V. L. VII
V. L. VII
T. V. L. VII
36.
D. L. VII
R. GEN
R. V. L. VII
V. L. VII.
37.
R. D. L. VII
R. GEN
R. V. L. VII
AU. G.
38.
R. GASS.
R. V. L. VII
R. D. L. VII
AU. G.
AU. VES.
39.
R. D. L. VII
R. V. L. VII
R. GEN.
R. AU.
AU. VES.
AU. G.
40.
N. C.
G.
Y.
MESEN.
EN.
41.
G.
MESEN.
Y.
EN.

EXPLANATION OF FIGURES

42 is taken five sections posterior to the auditory vesicle and lies just back of the point where the root of the IX later appears. The lateral mass is here completely converted into mesectoderm except the ventral portion (Y).

43 is taken through a condensation of lateral mass cells at the point where the root of the X and the jugular ganglion later appear. This mass cannot be located in later series for some time.

44 to 52 illustrate the detachment of the postauditory placode from the auditory vesicle, its migration and the formation of the lateralis X ganglion.

44 and 45 (A. neb., Stage IV) are consecutive sections. Fig. 44 being taken just at the point where the auditory vesicle passes into the placode by the disappearance of the dorsal half of the vesicle. Fig. 45 being near the anterior end of the placode, three sections posterior to the auditory vesicle.

46 is taken three sections posterior to the auditory vesicle and is from a slightly less developed embryo of the same age as figs 44 and 45. The vesicle passes gradually into the placode in this series.

47 to 51 (A. neb., Stage VII) show the characteristic appearance of the lateralis X ganglion as it is proliferated from the placode before the placode has moved beyond the posterior limit of the ganglion. Fig. 47 is twenty sections posterior to the vesicle; fig. 48 twenty-three sections; fig. 49 twenty-five sections; fig. 50 twenty-seven sections; and fig. 51 twenty-nine sections posterior to the auditory vesicle. Fig. 50 is at the extreme posterior limit of the lat. X ganglion.

52 (A. neb., Stage IX) shows the usual appearance of the lateralis X ganglion in a somewhat older series.

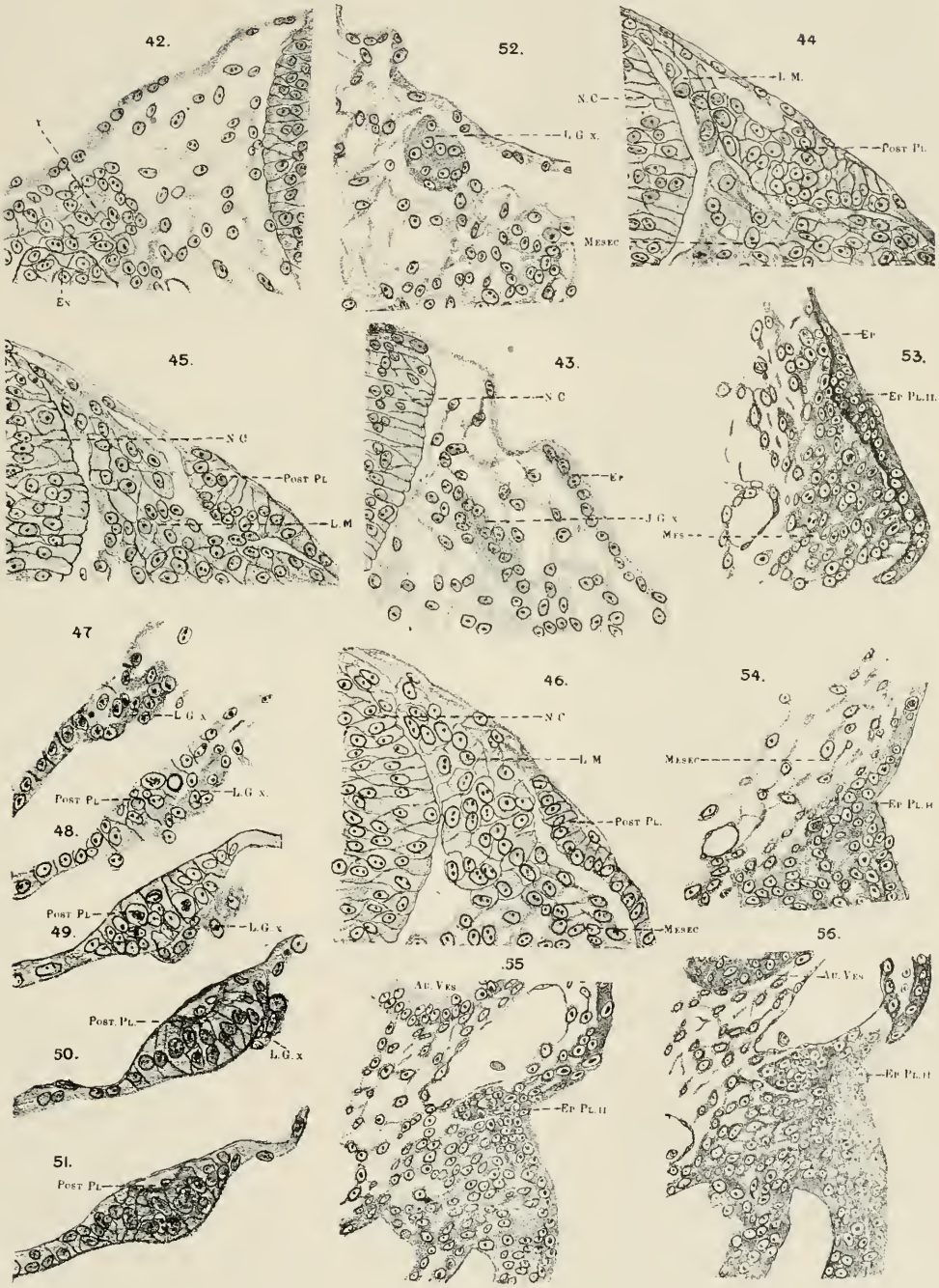
53 to 60 (A. melas) illustrate the formation and detachment of the epibranchial placode of the IX ganglion.

53 (A. melas, 56½ hours) shows the appearance of the placode when it can first be detected.

54 (A. melas, 69 hours) shows the extent of the thickening of the placode while the whole placode is still in contact with the epidermis.

55 to 59 are from the same embryo (A. melas, 81 hours) at a stage when the posterior end of the placode is detached from the epidermis and has formed an attachment to the root of the IX nerve which contains motor fibers and lateralis ganglion cells.

55, 56 and 57 are consecutive. Figs. 55 and 56 show the appearance of the ganglionic mass just before it becomes completely detached. The anterior end of the ganglion is attached, for some time after the posterior end is free, apparently by the growth dorsally and mesially of the ganglionic mass.


42.
En
52.
N C
L G x.
Mesec
44
L M.
Post Pl.
45.
N C
Post Pl
L M
43.
N C
Ep
L G x
Mes
53.
Ep
Ep Pl. II.
47
L G x.
Post Pl
L G x.
48.
Post Pl
49.
L G x
Post Pl.
50.
L G x
51.
Post Pl
46.
N C
L M
Post Pl.
Mesec
54.
Mesec
Ep Pl. II
55
Au. Ves
Ep Pl. II
56.
Au. Ves
Ep Pl. II

EXPLANATION OF FIGURES

57 is taken one section posterior to fig. 56 at the point where the ganglionic mass is free from the epidermis.

58 and 59 are taken through the root of the epibranchial ganglion of the IXth just before it comes into contact with the lateralis and motor portions of the IX, as shown in fig. 59.

60 (A. melas, 93 hours) is taken through the epibranchial ganglion at the nearest point of approach to the epidermis. This is the first stage in which the ganglion is completely detached from the epidermis. Its clean cut boundaries during all of its development indicate that no other cells than those derived from the placode are incorporated in the ganglion.

61, 62 and 63 (A. neb.) illustrate three stages in the formation of the lateralis IX ganglion of which fig. 63 (A. neb., Stage V) is the earliest. The ganglion varies a great deal in appearance and somewhat in position. Fig. 62 (A. neb., Stage IX) is older than fig. 61 (A. neb., Stage VIII). Fig. 63, while taken from the posterior end of the vesicle, differs totally from the appearance of this region before and after the formation of the ganglion.

64 to 71 show the formation of the first two epibranchial ganglia of the X.

64 (A. melas) 69 hours shows the earliest recognizable trace of the third epibranchial placode. Figs. 65 to 71 are from the same embryo (A. melas, 93 hours).

65 is through the anterior portion of the placode.

66 is through the middle region just before the detached portion is reached.

67 is just backof the point of detachment.

68 is through the root of the third epibranchial ganglion and the fourth epibranchial placode. This probably is not the earliest trace of the fourth epibranchial placode, however. The preceding series is defective at this point.

57.
Ep. Pl. II
Ep
58.
Au. Ves.
Ep. G. II
59
L. IX
Au. Ves
60.
Ep. G. II
63.
Au. Ves.
L. IX.
64.
Ep. Pl. III
61.
Au. Ves
L. IX
62.
Au. Ves
L. IX.
67.
Ep. G. III.
65.
Ep. Pl. III.
66.
Ep. Pl. III.
68.
Ep. G. III
Ep. Pl. IV

EXPLANATION OF FIGURES

69 (A. melas, 93 hours) is taken through the detached portion of epibranchial ganglion IV, also shows the root of epibranchial ganglion III.

70 (A. melas, 93 hours) is taken through the fourth epibranchial ganglion just before it comes into contact with the lateral mass ganglion of the X (general communis X). In fig. 70 the root of the third epibranchial ganglion has become so attenuated that it cannot be recognized with certainty, alhough the two cells marked Ep. gl. III appear to be the posterior extension of this ganglion.

71 (A. melas, 93 hours) shows the point of union of the fourth epibranchial ganglion with the lateral mass of the X (Gen. vis. X).

70, 72, 73 and 74 illustrate the conditions under which the fifth and sixth epibranchial placodes arise. In fig. 70 (A. melas, 93 hours) the fifth epibranchial placode appears before the lateral mass ganglion of the X comes into contact with the epidermis. In fig. 72 (A. melas, 99 hours) the attachment is quite like that in fig. 73 (A. melas 105 hours). In both these cases the attachment of a lateral mass (neural crest) ganglion to the epidermis is quite evident and corresponds to the oft repeated descriptions in the literature of the contact formed between neural crest ganglia and the epidermis. Nothing resembling this occurs in Ameiurus except in the fifth and sixth epibranchial placodes.

74 (A. melas, 113 hours) is taken through the attachment of the general communis X to the sixth epibranchial placode.

75 to 78 illustrate the formation of the jugular ganglion of the Xth. Fig. 75 (A. melas, 69 hours) shows the slight condensation of cells in the future position of the jugular ganglion before the appearance of the root of the X.

76 (A. melas, 81 hours). The cells which later form the jugular ganglion inclose the fibrous root of the X.

77 (A. melas, 113 hours) shows the first decided increase in size of the jugular ganglion when it begins to mass itself into definite areas of cells such as appear in fig. 78 (138 hours) where the ganglion is broken into small masses by the fibrillated root of the X.

69.

70.

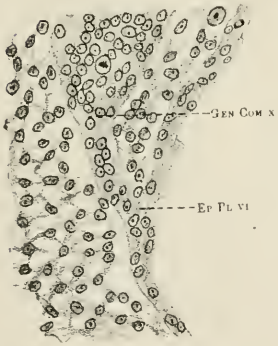
73.

71.

72.

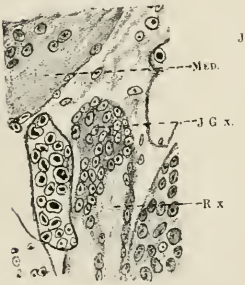
76.

74



75.

77.



78.

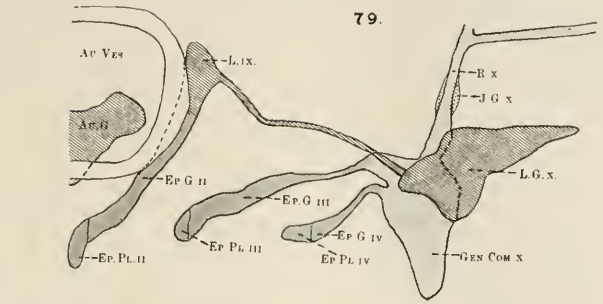
EXPLANATION OF FIGURES

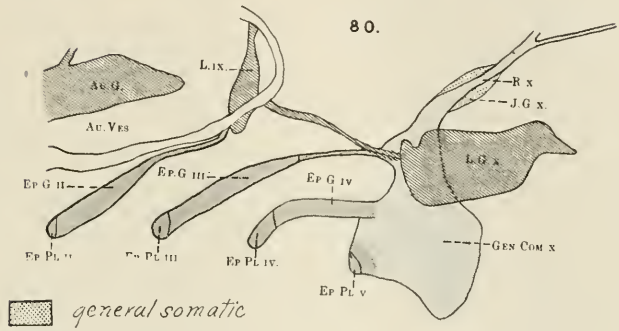
79 to 83 are reconstructions of the cranial ganglia of A. melas and are true in two dimensions, the anterior-posterior and the dorso-ventral. The reconstructions were made by projecting the section of the ganglion on paper to determine the vertical length of a given section. The lens of the eye, which is an almost perfect circle in these diameters, was used as a basis for determining the ratio of longitudinal to vertical dimensions. The reconstructions give an approximately exact picture of the lateral view of the ganglia as seen on a flat surface. The figures give no idea of the relative thickness of the ganglia and many of the roots appear as large as the ganglia while in reality they are quite thin, sometimes not more than one cell thick, cf. the roots of the II, III and IV epibranchial ganglia, figs. 79, 80 and 81. The object in making these reconstructions was to trace the embryonic ganglia up to a stage where they could be positively identified as the definitive ganglia of the adult.

79 is a reconstruction of the ninth and tenth ganglia of A. melas, 81 hours; only three epibranchial placodes are present at this stage.

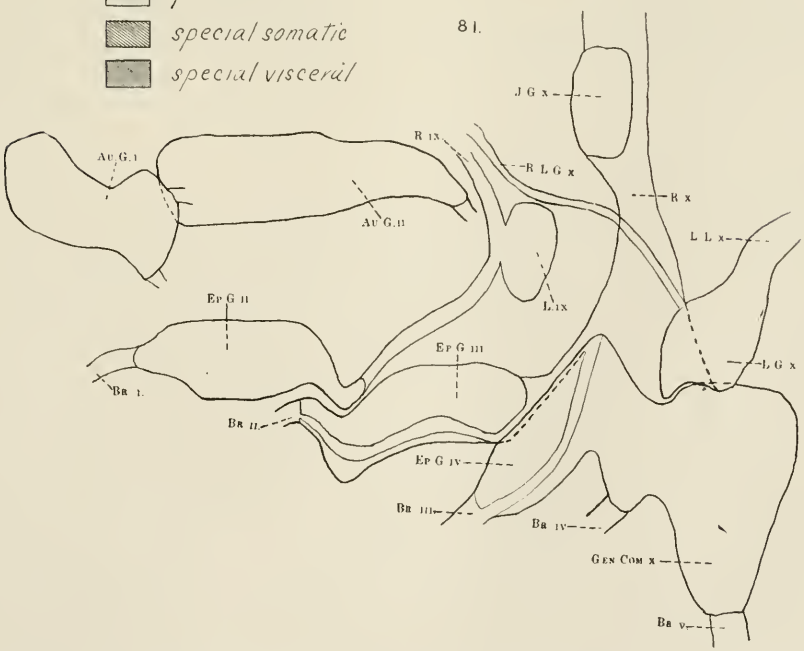
80 is a reconstruction of the ninth and tenth ganglia of A. melas, 93 hours. Four epibranchial placodes are here present.

81 is a reconstruction of the eighth, ninth and tenth ganglia of A. melas 138 hours. All placodes have disappeared some hours previous to this stage.


79.
Au Ves
L.IX.
R X
J G X
Au.G
Ep G II
Ep G III
L G X.
Ep G IV
Ep Pl III
Ep Pl IV
Ep.Pl.II
Gen Com X


80.
Au.G.
L.IX.
R X
J.G X.
Au.Ves
L.G X
Ep G II
Ep.G III
Ep G IV
Gen Com X
Ep Pl IV.
Ep Pl V

general somatic
general visceral
special somatic
special visceral


81.
J G X
R IX
Au.G.I
R L G X
R X
Au.G.II
L L X
Ep G II
L.IX
Ep G III
L G X
Br I.
Br II
Ep G IV
Br III
Br IV
Gen Com X
Br V

EXPLANATION OF FIGURES

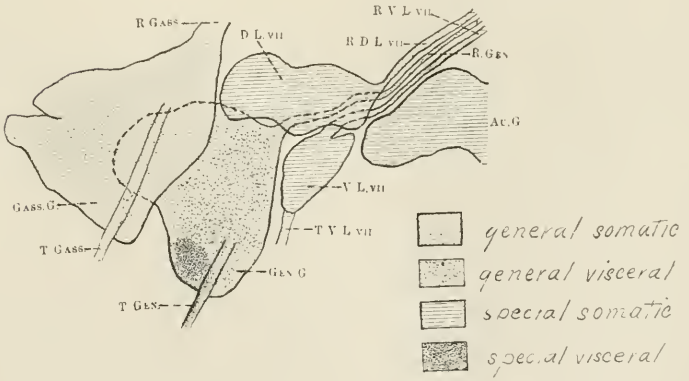
82 is a reconstruction of the eighth, ninth and tenth ganglia of A. melas, 174 hours.

83 is a reconstruction of the fifth, seventh and anterior portion of the eighth ganglia of A. melas, 86 hours. The roots of the V and VII ganglia are slightly schematized beyond their origin from the ganglia for the sake of clearness.

82.

R X
J G X
R IX
R L G X
AC G II
AC G I
L L V
R C D X
R A E
R P
L IX
R L
R N.
R S IX
R U I
R. A A
R Sac
L G X
EP G II
GEN COM X
BR I
BR II
EP G III
BR III
EP G IV
BR IV
BR V

83.


R GASS
D L VII
R V L VII
R D L VII
R GEN
AC. G
V L VII
GASS. G
T V L VII
T GASS
GEN G
T GEN
general somatic
general visceral
special somatic
special visceral

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IN THE JOURNALS PUBLISHED BY

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(APRIL 1910)

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THE MORPHOLOGY OF THE FOREBRAIN IN AMPHIBIA AND REPTILIA

C. JUDSON HERRICK

From the Anatomical Laboratory of the University of Chicago

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INTRODUCTION

Notwithstanding the publication of a great mass of descriptive detail regarding the structure and morphology of the forebrains of lower vertebrates, it is very difficult to form a clear picture of the fundamental morphological features of the vertebrate cerebral hemisphere. This wealth of observation has stubbornly resisted correlation and the morphological fruits of these arduous labors have until very recently, it must be confessed, been disappointingly meager. We have, however, now reached a point where effective correlation has begun to take form and within the bewildering complexity of detail characteristic of individual species it is possible to see a common morphological pattern which is sur-

prisingly constant and very simple. In this contribution I have brought together certain features of embryonic and adult brains of amphibians and reptiles which illustrate the fundamental simplicity of this pattern

Johnston has recently presented strong evidence that the telencephalon must be regarded as the terminal segment of the neural tube, a view confirming (with some modifications) the original teachings of His as opposed to the usage in the BNA tables In the latter the diencephalon is regarded as extending to the extreme rostral end of the primary neural tube, thus comprising the whole of the unpaired ventricle of this part of the brain and its lateral walls, including the lamina terminalis, while the telencephalon is regarded as limited to the secondarily evaginated parts of the neural tube termed the cerebral hemispheres, viz., the lateral ventricles and their massive walls. The usage of His and Johnston implies that the rostral part of the third ventricle, bounded behind by the velum transversum above and the chiasma-ridge (Johnston) below, and its walls are to be regarded as telencephalon medium, while the evaginated hemispheres constitute the telencephalon laterale. Johnston has further shown that in lower vertebrates there has been a progressive tendency as we pass up the developmental series (both ontogenetic and phylogenetic) for more and more of the telencephalon medium to be evaginated through the interventricular foramina into the hemispheres.

These considerations have an obvious bearing on the problem of the relation of the cerebral cortex to the primordial tissues from which it has been differentiated. With a view to the contribution of further data for the solution of this problem, I have examined the embryonic and adult brains of a series of types of lower vertebrates, the first results of which are presented in this paper.

I shall discuss the brains of fishes only incidentally and devote my attention chiefly to the amphibians and reptiles, whose cerebral hemispheres have evaginated so far from the primordial neural tube as to present a form approximating more closely to the mammalian conditions and readily leading up to them.

Our immediate problem, then, is the relations of the first recognizable primordia of the cerebral cortex to the other elements of the evaginated cerebral hemisphere and of all of these structures to the more ancient tissues of the telencephalon medium and diencephalon.

My indebtedness to the published works of Johnston will be evident to the reader throughout this paper. I have received still greater assistance from many extended conferences with Professor Johnston, in which he has freely shared with me his unpublished observations and stimulatng suggestions. The full extent of this obligation it is not necessary, nor indeed possible, for me to indicate here. It should, moreover, be added that, while many parts of this discussion have been greatly influenced and I trust improved by these conferences, the responsibility for the morphological views here expressed is wholly my own.

AMPHIBIA

I have studied an extensive series of sections of larval and adult Amblystoma, Necturus and various species of frogs, prepared by different methods, including the silver methods of Golgi and Ramón y Cajal, the method of Weigert, a toluidin blue modification of Nissl's method and various general embryological methods. Most of this material, except the larval Necturus which I studied through the courtesy of Professor Minot in the Harvard Embryological Collection, was prepared by Mr. P. S. McKibben of the Department of Anatomy, University of Chicago, to whose kindness and skill I am greatly indebted. I have also examined a series of cross sections through the head of Petromyzon (Ichthyomyzon concolor) prepared and kindly loaned to me by Dr. Charles Brookover.

In the Amphibia the wall of the cerebral hemisphere is naturally divided into five parts. Not to prejudice the morphological significance of these parts at the start, I shall call them simply olfactory bulb, ventro-medial, ventro-lateral, dorso-lateral and dorso-medial parts. They are especially distinct in the adult frog and are termed by Gaupp respectively lobus olfactorius,

eminentia septalis, ganglion basale, formatio pallii lateralis and eminentia pallii medialis. The two ventral parts are Gaupp's pars inferior s. subpallialis hemisphaerii and the dorsal parts are his pars pallialis (Gaupp, '99, fig. 29, p. 107). The dorsal and ventral parts are sometimes separated by well marked total fissures or by a conspicuous difference in internal structure. The fissure which separates the two lateral parts is the sulcus limitans lateralis of Gaupp and is incompletely homologous with the fissura endo-rhinalis (Turner) of other classes of vertebrates. The two medial parts of the frog are also separated by a total fissure, the fissura septo-corticalis of Kappers. Gaupp names this fissure the sulcus intermedius on the ventricular side and fissura arcuata on the superficial side. For reasons which will appear in the subsequent discussion, I name it fissura limitans hippocampi. The positions of both fissures in the frog are marked internally by a characteristic disposition of cells and fibers termed the zona limitans (lateralis et medialis), and the zona limitans may be present as a useful landmark in cases where the corresponding fissure is not externally evident.

URODELA

Amblystoma

We will first examine a series of transverse sections through the brain of a specimen of larval Amblystoma 17 mm. long and about 35 days of age after fertilization. The relations are very similar to those of the larvae of Necturus described by Warren ('05, 18 mm. stage) and by Kupffer ('06, 24 mm. stage) though with a less pronounced flexure in the thalamus region.

At the level of the olfactory bulb (fig. 1) the wall of the hemisphere is massive on all sides and the five parts as defined above are not separate. Secondary olfactory fibers (tractus olfactorius) are present around almost the whole periphery. The olfactory bulb is limited to the lateral aspect of the hemisphere.

A short distance caudal to the bulb (fig. 2) the greater part of the medial wall becomes membranous. This septum ependymale separates the dorso-medial from the ventro-medial part of

the hemisphere, both of the latter being relatively small. The medial olfactory tract divides into a dorsal part passing into the massive dorso-medial wall and a ventral part for the medial wall below the septum ependymale. The former joins the fimbria complex. The lateral olfactory tract is also divided into dorsal and ventral parts, the former (*tr. olf. lat.*) running separately to the pars dorso-lateralis of the hemisphere and the ventral being confused with the lateral forebrain tract and ending in the pars ventro-lateralis. The tract marked *tr. o.* is a mixture of the ventral parts of both the medial and lateral olfactory tracts and the medial and lateral components of the basal forebrain bundles, all of which are separate in the frog.

Midway of the hemisphere the septum ependymale is interrupted by the interventricular foramen and from this point caudad the ventriculus lateralis communicates widely with the ventriculus impar of the forebrain (fig. 3) The wide membranous roof over the ventriculus impar is a choroid plexus which is extended laterally to form the plexus lateralis Farther caudad this membrane is evaginated dorsally to form the paraphysis and backward into the third ventricle as velum transversum and diencephalic plexus, both of which at this age are very small. The line of contact of the roof membrane with the massive dorso-medial wall of the hemisphere is the taenia fornicis. The caudal border of this membrane is attached to the thalamus along the taenia thalami, where for a short distance the taenia fornicis and the taenia thalami come together (fig. 4). At the line of contact of the hemisphere and the thalamus there is a membrane stretching across from the taenia fornicis et thalami of one side to the other. This is the locus of the velum transversum (fig. 4). Passing forward from this point are three separate membranes: (1) the forebrain roof and the plexus lateralis already described; (2) the paraphysis (figs. 2 and 3); (3) the plexus chorioideus ventriculi tertii or diencephalic plexus. For further details regarding these membranes I refer to the excellent account of Necturus by Warren ('05).

At the levels of figs. 2 and 3 the pars ventro-medialis is very small with no recognizable pre-commissural body (nucleus media-

nus septi), the two lateral parts are imperfectly separated by a shallow ependymal groove which is the precursor of the fissura endorhinalis, and the dorso-medial part is sharply inflected from the dorso-medial angle to the taenia fornicis. The pars dorso-medialis becomes the primordium hippocampi of the adult.

The rostral ends of all four parts of the hemisphere are reached by fibers from the olfactory bulb and fibers of the tractus olfactorius dorso-lateralis follow the whole length of the pars dorso-lateralis and reach the posterior pole. The basal forebrain bundle contains two chief components which characterize respectively the ventro-lateral and the ventro-medial parts of the hemisphere, as seen best in the adult frog. The mingling of these components in urodeles is correlated with the imperfect separation of the two ventral parts of the hemisphere. The lateral and medial forebrain tracts can, however, be distinguished in Cajal preparations by the presence of much coarser fibres in the former (fig. 3). The medial forebrain tract connects chiefly with the hypothalamus, the lateral with the thalamus and mid-brain. Both decussate partially in the anterior commissure (fig. 5).

The taenia fornicis is accompanied by a mixed fiber tract whose composition varies in different parts and which I term the fimbria complex. At the rostral end it receives the dorsal component of the tractus olfactorius medialis (figs. 1 and 2) and comparison with the adult and with Anura suggests that here also it probably contains elements of the primordial columna fornicis system passing rostrally of the interventricular foramen and anterior commissure between the primordium hippocampi and the pars ventro-medialis. Farther back this tract contains fibers for the commissura pallii anterior (com. hippocampi), others for the stria medullaris and perhaps thalamic connections, all of which leave the primordium hippocampi in the region of the posterior pole caudal to the foramen interventriculare (see pp. 427 ff.).

The dorso-medial and dorso-lateral parts of the hemisphere converge at the posterior pole, where their distinctive characteristics are lost. This applies to the adults also of both Urodela and Anura.

The relations of telencephalon and diencephalon are difficult

to determine from sections in these specimens, on account of the strong diencephalic flexure. The caudal lip of the velum transversum passes over into the diencephalic plexus and, farther laterally and ventrally, into a ridge which forms a massive connecting bridge between the thalamus and the hemisphere which I shall term the eminentia thalami. This is bounded dorsally and ventrally by sharp ependymal grooves (fig. 5) which converge anteriorly to the interventricular foramen. The dorsal one of the these I shall term the sulcus diencephalicus medius, the ventral one the sulcus diencephalicus ventralis. The sulcus medius extends caudad through the whole length of the diencephalon, turning ventrally behind to join the sulcus limitans in front of the tuberculum posterius. As we shall see beyond (pp. 431 and 469) the sulcus medius is functionally an extension of the sulcus limitans (which ends in the preoptic recess), and the two sulci together in the diencephalon are sometimes termed sulcus Monroi. Kupffer ('06, p. 181, fig. 193) designates in Necturus the sulcus ventralis as sulcus Monroi, but this is evidently inappropriate, for this ventral sulcus separates the hypothalamus from the eminentia thalami (see pp. 431 and 469 ff.). Immediately behind the anterior commissural ridge in Amblystoma the ventral sulcus divides. One part follows the caudal border of the ridge into the preoptic recess. This is the ventral part of Kupffer's sulcus interencephalicus anterior (cf. '06, p. 175, fig. 187). The other part continues caudad dorsal to the preoptic nucleus and chiasma ridge to terminate blindly in the caudal part of the hypothalamus. It marks the boundary between the preoptic nucleus and hypothalamus and the thalamus in front and at its posterior end separates the hypothalamus proper from the tuberculum posterius, which probably belongs morphologically with the pars ventralis of the mesencephalon.

Still farther dorsal is a short sulcus extending (morphologically) caudad and dorsad from the interventricular foramen and forming the ventral boundary of the habenula. It is the sulcus diencephalicus dorsalis.

These relations come out much more clearly in older larvae and adults after the straightening of the diencephalic flexure.

They conform in morphological type to those figured by Sterzi in Acanthias ('09, p. 577, fig. 232).

Sections of much younger Amblystoma larvae of 10 mm. (about 15 days) so oriented as to cut the brain horizontally in the region of the velum transversum (figs. 6 and 7) illustrate clearly the relations of the velum (*v*) to the eminentia thalami and adjacent parts and the communication of the diencephalic and telencephalic ventricles through the wide aula in front of the velum.

The only massive connection between the hemispheres and the diencephalon at this age is by way of the eminentia thalami or structures lying farther ventrally which connect the ventral parts of the hemisphere with the ventral nuclei of the thalamus below the sulcus medius. The dorsal parts of the hemisphere are not well differentiated at this age, but comparison with older stages of Amblystoma and other Amphibia furnishes abundant confirmation of the statement last made.

The sections figured are so inclined as to show clearly that the connection between the dorsal part of the posterior pole and the wall of the thalamus is at this age wholly membranous (figs. 6 and 7, *z*). That is, it is comparable with the posterior chorioidal fold which is so conspicuous a feature of the brains of all embryonic reptiles and mammals. The fact that mitotic figures are more abundant around this angle of the lateral ventricle than elsewhere suggests that this is the point of most rapid growth at this age. In immediately following stages this fold is all incorporated into the extensive lateral plexus whose earliest rudiment is seen in fig. 6 at *y* (cf. fig. 3) and the massive tissue of the posterior pole rests in immediate contact with the eminentia thalami (figs. 3–5). There is at no stage in any of the Amphibia which I have examined a direct massive connection between the dorsal parts of the cerebral hemisphere and the pars dorsalis thalami (dorsally of the sulcus medius) or the epithalamus. Here, as in Anniota, the important fibrous connections between these dorsal parts of the telencephalon and diencephalon all cross the dorsal barrier interposed by the velum transversum and di-telencephalic fissure in the massive ventral parts. See p. 430 and the discussion on pp. 474 and 486.

The following description is based upon Amblystoma larvae between 30 mm. and 40 mm. in length, specimens taken at the time of metamorphosis and adults.

In all of these specimens the olfactory bulb (consisting of the glomeruli, mitral cells and granule cells) is confined to the lateral and extreme rostral parts of the hemisphere. In reading a series of cross-sections backward, as soon as the lateral ventricle appears the olfactory bulb is found to lie wholly laterally of it; but rostral to this level the layer of granule cells borders the whole median surface of the section. This short region where the granular layers of the two hemispheres are closely approximated (fig. 8), marks the site of the interbulbar union of the anuran brain. This figure from an adult brain shows medullated and unmedullated fibers from the mitral cells passing through the granular layer to accumulate on the median border of the hemisphere (fig. 9). Fibers of the tractus olfactorius medialis at the levels of these figures come from both the dorsal and ventral parts of the olfactory bulb, but farther caudad from the ventral part only.

In the adult at the rostral end of the olfactory bulb the mitral cells are separated from the glomeruli by a wide molecular layer. There are clusters of cells among the glomeruli which correspond with the subglomerular cells of Rubaschkin's description ('03) and probably with the periglomerular neurones of Cajal. Farther caudad the mitral cell layer is less compact and its cells spread throughout the molecular layer.

Beginning at the rostral tip of the lateral ventricle (fig. 9) the median wall of the hemisphere is occupied by an extensive and undifferentiated secondary olfactory nucleus, which I term the nucleus olfactorius anterior, and which as we pass caudad spreads through the medial and dorsal walls (fig. 10), the olfactory bulb occupying the whole ventro-lateral wall. Medullated and unmedullated secondary olfactory fibers pass from the olfactory bulb into the dorsal border of the anterior olfactory nucleus and continue caudad in this relation as tractus olfactorius dorso-lateralis. In a similar way medullated and unmedullated fibers curve around the ventral angle of the lateral ventricle to form the dorsal and ven-

tral divisions of the tractus olfactorius medialis. The ventral medullated tract arises only from the rostral end of the bulb. All of its fibers, which are few in number, terminated soon in the nucleus olfactorius anterior. The extent of distribution of the unmedullated fibers I have not determined, for the dorsal division enters the rostral end of the primordium hippocampi where its fibers are mingled with those of the fimbria complex and the ventral division is mingled with the median forebrain tract.

The tractus olfactorius dorso-lateralis arises from the whole length of the dorsal border of the olfactory bulb. The total number of medullated fibers is, accordingly, quite large (figs. 8 to 11). These medullated fibers are, however, all short, ending in the adjacent gray of the nucleus olfactorius anterior and pars dorso-lateralis of the hemisphere. The medullated tract does not increase in size as we approach the caudal end of the bulb and all its fibers terminate a short distance farther caudad (fig. 12). The accompanying unmedullated fibers doubtless extend farther caudad and reach the posterior pole as in the larva and the Anura, though my preparations do not demonstrate this in adult Amblystoma.

The tractus olfactorius ventro-lateralis arises from the caudal end of the olfactory bulb (corresponding with the bulbulus accessorius of the frog) and, as in the frog, passes directly back close to the ventricular ependyma to end in a cellular thickening at the caudal end of the pars ventro-lateralis opposite the anterior commissure, which corresponds with the so-called corpus striatum of the frog.

As we approach the caudal end of the olfactory bulb (fig. 11) the medial wall of the hemisphere becomes specialized into two structurally defined regions, the primordium hippocampi above and the nucleus post-olfactorius below, the latter corresponding to the eminentia post-olfactoria (Gaupp) of the frog brain and probably to the tuberculum olfactorium of mammals. The dorsal wall remains undifferentiated and is continued caudad into the pars dorso-lateralis of the hemisphere. The latter is, accordingly, to be regarded as the direct continuation of the nucleus olfactorius anterior.

The dorso-medial wall of the hemisphere from this point caudad to the posterior pole has the same structure and fiber connections as in the frog. Its cells are not arranged, as elsewhere in the hemisphere, in the form of primitive ventricular grey, but are scattered uniformly through the substance of the wall. Though no true cortex is formed here, the comparative anatomy of this part makes it plain that the cortex hippocampi of Amniota is differentiated within this region. Accordingly it is properly termed primordium hippocampi. From its rostral end a few medullated fibers accumulate close to the medial surface and descend to the ventro-medial angle, where they turn caudad and accompany the ventral forebrain tract to the hypothalamus (fig. 11). This tract is present also in adult Necturus and here, though the number of medullated fibers is still less than in Amblystoma, they run more separate from the other medullated fibers in the ventral forebrain tract so that the whole course can be read with ease. This is clearly the columna fornicis (see fig. 22). Many unmedullated fibers accompany this medullated tract between the primordium hippocampi and the pars ventromedialis hemisphaerii, but whether any of these extend to the hypothalamus and thus form a part of the columna fornicis is not clear. Similar unmedullated fibers are abundant between the primordium and the nucleus medianus septi for the whole extent of the latter. They are probably for the most part short association fibers. There is also a thin superficial layer of association fibers running around the dorsal angle of the hemisphere between the lateral and medial parts. This extends the whole length of the hemisphere. Accompanying the columna fornicis are many unmedullated fibers and a few medullated fibers between the pars ventro-medialis of the hemisphere and the hypothalamus, the whole complex being the medial forebrain tract.

Extending caudad from the olfactory bulb is the pars ventrolateralis hemisphaerii. This contains, in addition to the tractus olfactorius ventro-lateralis already referred to, medullated and unmedullated ascending and descending fibers between the hemisphere and the thalamus, the lateral forebrain tract.

A cross-section taken behind the olfactory bulb of adult Am-

blystoma resembles closely a corresponding section of the frog, save for the absence here of a clearly defined zona limitans lateralis (fig. 12). The pars ventro-medialis is thickened by the enlarged nucleus medianus septi.

As we approach the foramen interventriculare the medial wall below the primordium hippocampi, containing the nucleus medianus septi, becomes thinner (fig. 13) in the site of the larval septum ependymale, and for about 100 micra immediately rostral to the foramen the larval septum ependymale is preserved (fig. 14). No cells of the nucleus medianus extend caudad above the foramen.

The wide larval septum ependymale is almost obliterated by the growth into it from below and from in front of cells of the nucleus medianus septi. In larvae of 35 mm. this movement is in process, as shown by fig. 24, which illustrates a cross-section taken in a plane corresponding to fig. 13 of the adult.

At the level of the interventricular foramen the pars ventro-medialis is reduced in size and no clearly defined nucleus medianus septi is here present (fig. 15) in either larvae or adults. The pars dorso-medialis (primordium hippocampi) is in this region large and well differentiated in the adult, but in young larvae it is smaller than at its rostral and caudal ends. Compare fig. 15 (adult) with fig. 4, a section of the 17 mm. larva taken from the same part of the hemisphere. A corresponding section from a larva of 35 mm. is substantially the same as that of the 17 mm. specimen. The ontogeny shows, in fact, that the primordium hippocampi develops wholly within the dorso-median wall of the hemisphere and that its histological differentiation begins at its rostral and caudal ends, the middle part becoming differentiated at a later stage. The morphological significance of this fact will be commented upon in the discussion on page 487. The composition of the fimbria complex is as already described for the 17 mm. larva (p. 418). It receives secondary olfactory fibers from the tractus olfactorius dorso-medianus. The medullated columna fornicis fibers are confined to its rostral end. The unmedullated fibers passing between the primordium hippocampi and the nucleus medianus septi connect with all parts of the primordium in

the frog and probably also in Amblystoma. The fibers of the commissura pallii anterior (com. hippocampi) also reach all parts of the primordium.

Farther caudad in the fimbria complex the commissural fibers predominate. Unmedullated fibers of the commissura pallii anterior come in about equal numbers from the primordium hippocampi in front and from the posterior pole. The commissure passes down behind the interventricular foramen to cross in the dorsal part of the commissural ridge of the lamina terminalis (fig. 16). It is the "dorsal commissure" or the dorsal component of the anterior commissure of the older literature and the "corpus callosum" of Osborn and his followers.

The "dorsal commissure" and the ventral or anterior commissure are not so clearly separate in urodeles as in the frog and comparison with the latter type shows that some of the components of the dorsal commissure of urodeles are dissociated from it in Anura. Morphologically the dorsal commissure, or commissura pallii anterior, should be defined as containing only fibers which connect with the dorsal part (pars pallialis) of the hemisphere, and all fibers related only with the ventral parts of the hemisphere should be classed with the anterior commissure regardless of their topographic position in the commissural complex at the median plane.

In the region where the dorsal commissural fibers descend into the commissure ridge of the lamina terminalis an important component of the fimbria complex continues directly caudad to enter the stria medullatis of the same side. The relations here are very intricate, as will appear beyond. This connection lies dorsally of the interventricular foramen, but ventrally of the sulcus medius, *i.e.*, in the pars ventralis thalami, and it occurs in the same relations in Anura also. In Anmiota the configuration of the parts is so modified, chiefly by the posterior chorioidal fold, as to render such a connection impossible.

At the point where the taenia fornicis joins the taenia thalami the dorsal parts of the hemisphere are cut off from the pars dorsalis thalami and epithalamus by the di-telencephalic fissure and the dorsal plexuses (paraphysis, etc. For the relations of

these plexuses see beyond, p. 431). Here in the eminentia thalami, which borders the taenia (figs. 16 to 19), many fiber systems are crowded together.

The observed relations are these, the following tracts being all unmedullated except as specified. The fimbria complex, bearing a few medullated fibers (fig. 15) connected with the primordium hippocampi, divides into two parts, each with medullated fibers, one entering the commissura pallii anterior, the other the stria medullaris (fig. 16). Medullated fibers pass between both of these subdivisions and the adjacent grey of the pars ventro-lateralis hemisphaerii (striatum complex). Both subdivisions have unmedullated connections with the posterior pole. Farther back (figs. 18 and 19) the stria medullaris has an unmedullated connection with the eminentia thalami and a strong medullated and unmedullated connection with the rostral end of the pars ventralis thalami. Golgi preparations of adult Necturus show that some at least of the fibers between the stria medullaris and the pars ventro-lateralis hemisphaerii, pars ventralis thalami and eminentia thalami end in these parts by free arborizations. In view of the fact that these are all centers of efferent discharge, I interpret these fibers as conducting downward from the habenula to the somatic motor correlation centers.

There is also a strong connection between the stria medullaris and the preoptic nucleus, which is very large in urodeles. This tract runs chiefly external to the lateral forebrain tract but partly internal to it. A few medullated fibers are found in the latter path (fig. 19). This tractus olfacto-habenularis lateralis et medialis reaches as far forward as the pars ventro-medialis hemisphaerii and possibly as far back as the hypothalamus. Johnston describes in Necturus a tract from the "medial olfactory nucleus" (nucleus medianus septi) to the commissura pallii anterior. I find the tract in Necturus and in the frog in the same relations as figured by Johnston ('06, p. 306, fig. 150). Golgi preparations of adult Necturus show that these fibers arise chiefly from the bodies of cells bordering the recessus superior. These cells lie very close to the ventricle and look like ependyma cells, though probably they should not be classed as such. This tract doubt-

less occurs in Amblystoma also, though I have not been able to demonstrate it. Some of its fibers cross in the commissure, but most of them ascend by way of the stria medullaris to the habenula of the same side. It is therefore a component (partly decussating?) of the tractus olfacto-habenularis. I have not been able to find the "tractus lobo-epistriaticus" described by Johnston ('06, p. 309) as passing from the hypothalamus to the primordium hippocampi by way of a decussation in the commissura pallii anterior. These fibers may be the same as the ones which I interpret as tractus cortico-thalamicus, or I may have overlooked them.

On the basis of the relations just described and of the study of an extensive series of brains of Necturus and the frog I interpret the composition of the fimbria and stria medullaris systems of fibers in urodeles as expressed in the following summary (see also fig. 22).

1. The fimbria complex at its rostral end receives fibers of the tractus olfactorius dorso-medialis, is connected by association fibers (ascending and descending) with the nucleus medianus septi and gives rise to the columna fornicis. At its caudal end it contains fibers of the commissura pallii anterior, tractus cortico-habenularis medialis and tractus cortico-thalamicus. The hippocampal commissure is divided into two parts, the commissura pallii anterior and posterior.

2. The commissura pallii anterior includes commissural fibers between the dorso-medial parts of the hemispheres and decussating fibers of the tractus cortico-habenularis medialis and cortico-thalamicus.

3. The stria medullaris is a very complex tract bordering the taenia thalamia. It includes a part of the course of all of the following tracts.

4. The commissura pallii posterior. These are unmedullated fibers which arise from the ventral surface of the posterior pole of the hemisphere and pass directly medial-ward to join the stria medullaris, within which they ascend to the commissura superior and thence pass to the posterior pole of the other hemisphere. They are homologous with the similar tract described by Elliot Smith ('03) in reptiles under the name, commissura aberrans.

5. The tractus olfacto-habenularis system. Primitively, as in cyclostomes, this tract runs in diffuse formation from practically all parts of the secondary olfactory center to converge in the stria medullaris and reach the habenula, part of its fibers first decussating in the superior commissure. In the Amphibia, with the further differentiation of the caudal part of the telencephalon, the relations become very complex, though the same in principle. There are five components (paragraphs 6 to 10 below) of this system, of which the largest comes from the nucleus preopticus and constitutes the tractus olfacto-habenularis in the restricted sense.

6. The tractus olfacto-habenularis lateralis arises chiefly from the anterior part of the preoptic nucleus (see p. 432) and passes upward into the stria medullaris laterally of the lateral forebrain tract (fig. 19).

7. The tractus olfacto-habenularis medialis arises chiefly from the pars magno-cellularis of the preoptic nucleus and ascends internal to the lateral forebrain tract (fig. 18).

8. The tractus septo-habenularis arises in the nucleus medianus septi (it is much larger in Necturus) and passes backward close to the ventricular ependyma to cross the dorsal surface of the anterior commissure and enter the stria medullaris in company with the tractus cortico-habenularis medialis.

9. The tractus cortico-habenularis lateralis is one of the largest components of the stria medullaris. It passes from the posterior pole in company with the commissura pallii posterior (fig. 18).

10. The tractus cortico-habenularis medialis consists of a few fibers which pass from the caudal end of the primordium hippocampi directly into the stria medullaris. Some of its fibers probably cross in the commissura pallii anterior.

All of the components of the tractus olfacto-habenularis just enumerated (numbers 6 to 10) occur in the frog and all but the last in the reptiles.

11. Tractus cortico-thalamicus. This is a sparse collection of medullated fibers accompanying the tractus cortico-habenularis medialis to the stria medullaris; but instead of turning dorsally into the habenula, they continue backward into the thalamus

and apparently reach the pars ventralis thalami above and behind the optic chiasma. A part of this system decussates in the commissura pallii anterior. I have not been able to trace any of its fibers into the hypothalamus. This tract was recognized in the frog by P. Ramón y Cajal and named fornix longus. If any fibers of this tract reach the hypothalamus, these would be able to serve as an aberrant columna fornicis, connecting with the caudal part of the primordium hippocampi, just as the typical columna fornicis connects with its rostral end (see fig. 22); but neither my preparations nor P. Ramón's figure ('96, p. 249) give any evidence of hypothalamic connections.

12. Tractus habenulo-striaticus. Scattered medullated fibers leave the stria medullaris to spread through the grey matter of the caudal end of the pars ventro-lateralis hemisphaerii laterally of the anterior commissure This is the region designated corpus striatum by some recent authors Though not fully homologous with the mammalian striatum, it is one of the sources of that structure; accordingly I term the fibers tractus habenulo-striaticus.

13. Tractus habenulo-thalamicus. Similarly medullated and unmedullated fibers in larger number enter the rostral end of the pars ventralis thalami.

14. Tractus thalamo-habenularis. These fibers pass in diffuse formation between the pars dorsalis thalami and the habenula and the most rostral members of the group are for a short distance joined to the stria medullaris.

In the 17 mm. specimen we found the posterior pole of the hemisphere solid and extending but a short distance behind the level of the anterior commissure (fig. 5). At 32 mm. the same is true and the diencephalic flexure still obscures the relations between diencephalon and telencephalon somewhat (figs. 25 and 26). In the adult this flexure has disappeared and the posterior pole has grown far backward laterally of the thalamus (figs. 16 to 20). The primordium hippocampi forms the median wall of the posterior pole and the pars dorso-lateralis the lateral wall, but its caudal end is a relatively undifferentiated tissue which is in contact with the caudal part of the striatum complex below and which has the following characteristic fiber connections: tractus olfac-

torius dorso-lateralis on the lateral border, thalamic connections by way of the lateral forebrain bundle and striatum complex, commissural fibers in both the commissura pallii anterior and posterior, epithalamic connections by way of the stria medullaris.

The two dorsal parts of the hemisphere, then, terminate in the posterior pole. They do not directly connect with any diencephalic structures and their functional relations with the latter are all effected by tracts which cross the di-telencephalic fissure in the ventral part of the brain tube, *i .e.*, ventrally of the sulcus medius, either by way of the basal forebrain bundles or by way of the stria medullaris. Although the latter receives fibers directly from the caudal end of the primordium hippocampi dorsal and caudal to the interventricular foramen (a condition which does not prevail in mammals), nevertheless these connecting fibers pass through the eminentia thalami (figs. 17 and 18) which corresponds in position to the ventral part of the lateral thalamic wall (see pp. 476, ff) and not directly from dorsal telencephalic to dorsal diencephalic centers. We shall find that the same condition prevails in the frog, though the eminentia thalami is reduced there to a small vestige which is crowded far dorsally. This vestige in the frog is the nucleus supracommissuralis of the literature, which I shall term the nucleus of the commissura hippocampi. For further discussion of this nucleus see p. 440.

The ventro-median part of the hemisphere merges with the nucleus preopticus and this with the hypothalamus, these forming a continuous column of grey, with the median forebrain tract connecting all parts. This tract corresponds with the tractus olfacto-hypothalamicus medialis of fishes and contains both descending and ascending fibers.

The ventro-lateral part of the hemisphere is characterized by the lateral forebrain tract and is continued backward into the prominentia fascicularis (Gaupp) of the thalamus, this prominence being much more evident in the Anura than in urodeles. The coarse fibers of this tract are clearly seen to reach all parts of the ventro-lateral and dorso-lateral parts in the mid-region of the hemisphere; they also reach the posterior pole. A larger proportion of the fibers of this tract decussates in the anterior commissure

in these specimens than in the frog. The lateral forebrain tract is the great conduction path between the lateral parts of the hemispheres and the thalamus.

As is well known, the choroid plexuses of the region are very complex in adult urodeles; but their development in Amblystoma, Necturus (Warren, '05) and Salamandra (Kupffer, '06, p. 171) shows that the lateral plexuses, median telencephalic plexus (auliplexus, of Kingsbury, '95, fig. 4, and Fish, '95; fig. 3) and paraphysis *sensu stricto* develop from the telencephalic lamina of the velum transversum in the typical manner, while the diencephalic plexus arises from the caudal lamina of the velum. Fig. 16 is taken at the point of union of the median telencephalic and diencephalic plexuses, *i.e.*, at the dorsal boundary between telencephalon and diencephalon. The eminentia thalami is developed wholly caudal to this level. The di-telencephalic boundary curves downward and backward rostral to this eminence along the line of the sulcus ventralis in figures 17 to 21 to terminate far caudad behind the optic and post-optic commissure ridge. This very anomalous relation grows out of the exaggerated size of the preoptic and supraoptic nucleus in the telencephalon medium of urodeles.

The plan of the diencephalon appears with diagrammatic simplicity in cross sections of the adult brain, where the embryonic diencephalic flexure no longer complicates the matter. The three longitudinal sulci found in the 17 mm. larva (see p. 419) are preserved. The sulcus diencephalicus ventralis passes backward from the interventricular foramen and separates the preoptic nucleus and hypothalamus from the thalamus.

The sulcus medius passes backward from the foramen and separates the thalamus (medithalamus) into dorsal and ventral parts. It becomes less distinct farther caudad and, as in the larva, joins the sulcus limitans. The rostral end of the pars ventralis thalami is somewhat enlarged and separated from the remainder by a wide groove. This is the eminentia thalami (figs. 17, 18 and 22) which is larger in young larvae than in the adult.

The sulcus diencephalicus dorsalis separates the epithalamus from the thalamus. It is divided into two segments, the rostral,

one of which is the subhabenular sulcus which curves dorsally around the caudal end of the habenula. The other segment begins under the caudal end of the habenula and extends backward through the whole remaining length of the diencephalon. The tissue dorsally of it apparently corresponds with the "post-habenuläre Zwischenhirngebiet" described by Goldstein ('05) in teleosts. Its morphology is obscure. The pars dorsalis thalami does not extend as far rostrad as the pars ventralis. Accordingly, the sulcus medius in the rostral part of its course (figs. 18 and 19) separates the pars ventralis (eminentia thalami) from the epithalamus instead of from the pars dorsalis thalami, as farther caudad (see fig. 22). The sulcus diencephalicus dorsalis is interrupted by the commissura posterior, behind which the tectum mesencephali for a short distance lies dorsally of the thalamus in the same relations as the post-habenular intermediate region does farther forward (fig. 21). A short distance farther caudad the pars dorsalis thalami also disappears or is merged with the tectum mesencephali and the pars ventralis thalami passes over into the pedunculus cerebri region under the tectum.

The sulcus limitans extends forward from the midbrain into the preoptic recess, its whole diencephalic extent being preserved in the adult Amblystoma (see fig. 22). In the adults of some other amphibians it seems to be obliterated where it crosses the caudal end of the pars ventralis thalami. In fig. 22, which is drawn from sagittal sections of adult Amblystoma, there are seen two short sulci running ventrally from the interventricular foramen across the preoptic nucleus. The more rostral one follows the caudal border of the anterior commissura ridge and is the ventral part of Kupffer's sulcus interencephalicus anterior, as we found it in the 17 mm. larva. The other vertical sulcus divides the preoptic nucleus into rostral and caudal portions (figs. 18 and 19), which I shall term the pars anterior and pars magnocellularis of the preoptic nucleus. The latter is homologous with the nucleus magnocellularis of fishes and is intimately related with the corpus striatum complex and the rostral end of the pars ventralis thalami. The tractus olfacto-habenularis medialis arises chiefly from this nucleus, the tractus olfacto-habenularis lateralis from the rostral

part of the preoptic nucleus. I find the same relations in the frog.

The pars ventro-lateralis of the hemisphere contains elements which give rise to the corpus striatum of mammals. From it (and probably also from the pars dorso-lateralis) fibers arise which terminate in the pars ventralis thalami. This component of the lateral forebrain tract is the tractus strio-thalamicus, which partially decussates in the anterior commissure. Its fibers, which are partly medullated, terminate chiefly at the transverse level of the post-optic commissure, though some continue farther caudad. In the caudal part of the pars ventralis thalami they are joined by many other medullated fibers from this part to form the tractus thalamo-bulbaris et spinalis, and by the tractus mammillo-bulbaris from the hypothalamus. These fibers enter the strong ventro-lateral descending tract of the midbrain and oblongata. The tractus tecto-spinalis is added in the midbrain and the whole system evidently corresponds with the descending pedunculus cerebri tracts of mammals. From this it follows that the ventral part of the hemisphere and the ventral part of the thalamus are of common type, both being fundamentally centers of efferent discharge and the correlations directly connected therewith.

The great lemniscus system of the medulla oblongata passes forward into the dorsal part of the midbrain, *i.e.*, above the sulcus limitans, where the larger part of its fibers terminate in the tectum mesencephali (primordial colliculus inferior). This portion of the lemniscus of Necturus is shown in Kingsbury's figures ('95). Some of its fibers, however, continue upward to end farther forward in the tectum and in the pars dorsalis thalami. These are joined by other fibers from the tectum mesencephali to the thalamus. A strong medullated tract associated with the rostral end of the lemniscus passes from the tectum mesencephali through the lateral part of the thalamus to enter the post-optic commissure. Other similar fibers enter this commissure from nearly all portions of the pars dorsalis thalami.

A large collection of medullated and unmedullated fibers passes from the rostral end of the pars dorsalis thalami to the grey nuclei

in the caudal end of the pars ventralis thalami from which the tractus thalamo-spinalis arises. This is the tractus thalamo-peduncularis. An unmedullated tract passes from the rostral end of the pars dorsalis thalami into the hypothalamus, the tractus thalamo-mammillaris. A much larger tract of unmedullated fibers passes from the middle and rostral part of the pars dorsalis thalami to the lateral forebrain tract, and thence to the cerebral hemisphere. This is the precursor of the tractus thalamo-corticalis of mammals and may receive the same name here, though its termination in the hemisphere cannot be given more precisely than to say that it reaches its lateral parts.

The pars dorsalis thalami is evidently a receptive center for somatic (exteroceptive) impulses. These come from the spinal cord and oblongata by way of the lemniscus and from the tectum mesencephali by fibers associated with the lemniscus. Optic impulses are also received directly by collaterals from the tractus opticus ending in the corpus geniculatum laterale. This connection has been frequently described in fishes, where the geniculate body is very small. Johnston mentions it in Acipenser ('01, p. 57) though the lateral geniculate body is not here separable from the nucleus anterior of the pars dorsalis thalami, a condition which prevails to some extent in Amphibia also, where in young larvae the optic connection is confined to the posterior part of the thalamus. In adult Amblystoma the lateral geniculate body covers more than two-thirds of the pars dorsalis thalami and in the frog it has extended forward so as to cover almost its whole lateral surface. I have observed collaterals from the tractus opticus ending in relation with these cells. From this it follows that the pars dorsalis thalami receives optic fibers along with those of the other exteroceptive senses—tactile, somaesthetic and acoustic—received from the lemniscus, and that all of these sensory elements may be represented in the sensory radiations which enter the telencephalon from this part by way of the lateral forebrain tract.

From the preceding description it appears that in Amblystoma the massive lateral wall of the diencephalon on each side is divided longitudinally into four parts which bear simple and obvious rela-

tions to corresponding parts of the homolateral cerebral hemisphere. The development shows that these parts in both cases are differentiated from the primitive central grey of the early embryo by a more rapid cellular proliferation than occurs in the intervening sulci; *i.e.*, they are functionally defined. Accordingly, we find them related by important fiber tracts which clearly demonstrate the underlying functional motives of the differentiation.

The hypothalamus is continued forward directly into the pars ventro-medialis hemisphaerii, the whole column being an olfactovisceral center, the rostral end of which is receptive and the caudal end efferent.

The pars ventralis thalami is continued forward directly into the pars ventro-lateralis hemisphaerii, the whole column in both the telencephalon and diencephalon being the primary pathway of efferent discharge into the somatic motor centers.

The pars dorsalis thalami is the receptive center for somatic sensory (exteroceptive) impressions and is functionally related with the lateral wall of the hemisphere, probably (on comparative grounds) chiefly with its dorsal part.

The epithalamus corresponds in position with the pars dorsomedialis hemisphaerii (primordium hippocampi); but the morphological problems involved here are not as simple and self-explanatory as in the other cases and can best be discussed on a later page (p. 468, ff.).

Other Urodela

The brain of Desmognathus fusca, as described by Fish ('95) is very similar to that of Amblystoma. The figures show that both primordium hippocampi and nucleus medianus septi are less highly developed and the membranous septum ependymale is extensive. In these respects adult Desmognathus seems to be intermediate between the 17 mm. and the 35 mm. Amblystoma larvae. No medullated fibers were found in the cerebral hemisphere except in the lateral forebrain tract. The three diencephalic sulci are present as I have described them in Amblystoma,

their positions being indicated in the median dissection of the brain shown in Fish's fig. 3 by projections of the choroid plexus which lie within them.

In Amphiuma (Muraenopsis) the pars ventro-medialis is also feebly developed (Osborn, '83). There is no septum ependymale, the brain being greatly flattened dorso-ventrally. This brings the ventral border of the pars dorso-medialis far ventrad (fig. 23). A small precommissural body is seen around the ventro-medial angle of the lateral ventricle. The dorso-medial and dorso-lateral parts are separated by a deep ependymal groove, while the boundary between the two ventral parts is not so clearly marked.

The 10 mm. larva of Diemyctylus viridescens (Mrs. Gage, '93) in the matters here under discussion is very similar to the 17 mm. larva of Amblystoma, and the adult Diemyctylus to the adult Amblystoma.

In the brain of the adult Plethodon glutinosus the figures of Dodds ('07) show relations of the walls of the hemisphere which are similar in essential points to those of adult Amblystoma.

In none of the species which I have hitherto described do the cells of the nucleus medianus septi extend dorsally above the interventricular foramen.

Through the kindness of Dr. J. B. Johnston I have examined sections of the brain Cryptobranchus and find the relations very similar to those of Necturus. The nucleus medianus septi is larger than in Amblystoma and extends for a short distance backward dorsally of the interventricular foramen.

I have studied the conditions in adult Necturus and in larvae, the latter from specimens in the Harvard Embryological Collection. Kingsbury ('95) has given an excellent series of figures of the adult brain, and comparison of these with the preceding figures of adult Amblystoma will render a detailed discussion of the conditions in Necturus unnecessary.

In adult Necturus the septum ependymale has become massive except for a very short remnant immediately rostral to the interventricular foramen. The nucleus medianus septi is moderately developed in the ventro-median part of the hemisphere. As we approach the lamina terminalis, it is divided into a ventral and a

dorsal segment by the vestige of the septum ependymale, the dorsal segment being separated from the overlying primordium hippocampi by a deep ependymal groove which represents an incomplete fissura limitans hippocampi (see Kingsbury, '95, figs. 32 and 31). The nucleus medianus septi extends for a very short distance backward above the interventricular foramen, this part being homologous with the pars fimbrialis septi (Kappers, '08) of the frog. In my specimens of Necturus neither the eminentia thalami nor the diencephalic sulci are so distinct as in Amblystoma. The other features of this brain so far as noted agree substantially with those of Amblystoma.

The preparations in the Harvard Embryological Collection show that in the Necturus larva of 29.6 mm., as the nucleus medianus septi invades the septum ependymale, it pushes farther backward in the dorsal border of the septum than in the ventral. This is in contrast with the condition found in Amblystoma, as will be seen by a comparison of fig. 24 with fig. 27. In the latter figure the right side is farther caudad than the left, by reason of slight obliquity of the section. The nucleus medianus fills the whole septum on the left side, but on the right has grown back into its dorsal part only. This is the beginning of the pars fimbrialis septi. Two sections (28μ) farther back the septum is wholly membranous (fig. 28), and the interventricular foramen appears in the next section caudad.

In Kupffer's account ('06) of the larval Necturus 24 mm. long it is evident from the preceding considerations that he has incorrectly identified some of the structures in the median wall of the hemisphere. In his fig. 191 he designates rostrally of the interventricular foramen a sulcus intermedius said to be equivalent to the fissure so named by Gaupp in the frog and to separate the eminentia pallialis medialis (my primordium hippocampi) from the eminentia septalis (my pars ventro-medialis). This is impossible, for the part here marked eminentia septalis gives rise to the fibers of the hippocampal commissure and conforms in every other respect to the pars dorso-medialis and not to the pars ventro-medialis; *i.e.*, it is equivalent to the primordium hippocampi of the frog and not to the eminentia septalis, as Kupffer supposed.

My study of larval and adult Necturus shows clearly that Kupffer's eminentia pallii medialis corresponds with a part of the undifferentiated dorsal wall of the hemisphere and not with the eminentia pallialis medialis (Gaupp) of the frog; that his sulcus intermedius is a furrow within the pars dorso-medialis and does not correspond with the indentation so named by Gaupp in the frog, the latter being the sulcus limitans hippocampi of my description; and that his eminentia septalis is Gaupp's eminentia pallialis medialis and my primordium hippocampi. The ependymal sulcus designated sulcus intermedius by Kupffer in the larva is present in the adult (see Kingsbury, '95, figs. 29, 30, 31) in the same relations, separating the primordium hippocampi from an undifferentiated portion of the pars dorso-medialis. The sulcus limitans hippocampi (sulcus intermedius Gaupp) is seen ventrally of this in Kingsbury's fig. 31.

ANURA

In the half-grown frog tadpole, as in the adult, the olfactory bulbs are fused at their tips, the fusion involving the glomerular and mitral cell layers only. These layers extend to the extreme rostral end of the bulbs and are not confined to their lateral surfaces, as in urodeles and younger frog larvae.

As compared with the urodeles, the ventro-median part of the hemisphere is greatly enlarged and a dense accumulation of cells is found in its dorsal part. This is the precommissural body, or nucleus medianus septi (fig. 29). The region corresponding to the septum ependymale of urodeles is very massive and the lamina terminalis is greatly thickened (fig. 30). The nucleus medianus septi extends dorsal and caudal to the interventricular foramen, though not so extensively as in the adult (fig. 31). This is the pars fimbrialis septi of Kappers ('08).

The walls of the hemisphere are entirely massive rostrally of the interventricular foramen. Caudad, however, the roof is even more widely membranous than in urodele larvae. The foramen is very wide and, as in the adult, there is no plexus lateralis. The membranous roof of the forebrain ventricle is attached to the

massive wall of the hemisphere by the taenia fornicis which is directly continuous caudad with the taenia thalami. From this roof membrane the paraphysis and plexus chorioideus of the third ventricle are developed. The frog larva figured by Kupffer ('06) is apparently older than the specimens here figured and resembles the adult frog more closely.

The four fundamental parts of the wall of the hemisphere of the adult frog are here imperfectly separate, as in the urodeles. The cells of the dorso-median part have the diffuse arrangement characteristic of this region in the adults of both the frog and urodeles. The relations of the commissura pallii anterior to this region (fig. 32, *commis. hippocampi*) fix its homology as primordium hippocampi. In the region of the lamina terminalis and farther rostrad the primordium hippocampi is clearly separated from the precommissural body by a cell-free zona limitans (figs. 29 and 30). An ependymal sulcus in fig. 29 marks the position of the fissura limitans hippocampi of the adult, a fissure which is termed fissura arcuata by Gaupp, though incorrectly, as will appear beyond, for the latter fissure is absent in both larval and adult Amphibia.

The relations at the posterior pole are very similar in these larvae to those of Amblystoma of 30 mm. to 40 mm., described above. The lateral ventricle is extended into it for a short distance caudad of the lamina terminalis and the primordium hippocampi tissue is also extended farther caudad. The commissura pallii anterior is derived chiefly from the primordium hippocampi, but partly from the posterior pole, and at the point where it turns downward from the latter to pass behind the interventricular foramen into the lamina terminalis it comes in contact with the stria medullaris, as in urodeles. At this point of contact there is developed a small dense nucleus, which here bears the same relation to the taenia fornicis as does the caudal end of the pars fimbrialis septi farther rostrad (cf. figs. 31 and 32). These nuclei are, however, not continuous and are of quite different origins. This nucleus is termed by Gaupp in the adult frog nucleus supracommissuralis. I term it for reasons which will appear beyond the nucleus of the commissura hippocampi. In the tadpole it

is relatively larger than in the adult and increases rapidly in size farther caudad. It lies ventrally of the sulcus diencephalicus medius, is the equivalent of the eminentia thalami of urodeles and is directly continuous, as recognized by Gaupp ('99, p. 82), with the pars ventralis thalami (pars medialis, Gaupp). Its fiber connections I have not determined with certainty in the frog, but its uniform position at the union of the fimbria complex and the stria medullaris suggests that it is related to both of these tracts (see especially fig. 33), as indeed I find to be the case in reptiles. In the urodeles numerous fibers pass from the equivalent structure (eminentia thalami) into the stria medullaris (fig. 18). The fibers of the tractus cortico-habenularis medialis pass directly through it and some of these fibers may end here. The same is true of fibers of the tractus olfacto-habenularis from the preoptic nucleus.

Some of my preparations of adult frog brains by the silver reduction method of Ramón y Cajal show very delicate fibers passing between this nucleus and the commissura pallii anterior. These appear to be dendrites of the cells of the nucleus and collaterals of the commissural fibers. Golgi preparations of adult Necturus show clearly short collaterals from the commissural fibers coming into relation with the cells of this nucleus. This indicates that the nucleus constitutes, in part at least, a station for nervous impulses passing between the primordium hippocampi and the habenula. The fibers from the commissura pallii anterior (com. hippocampi) thus constitute a tractus cortico-habenularis medialis cruciatus, with a synapse interpolated in this path at the supracommissural nucleus. The crossed and uncrossed path together, in this case, are strictly comparable with the tractus cortico-habenularis of reptiles and mammals save that in Amphibia they pass (like the commissura hippocampi itself) dorsad and caudad of the interventricular foramen to reach the stria medullaris, whereas in Amniota the path goes rostrad and ventrad of the foramen. We shall find the relations of these tracts in reptiles very instructive in this connection. (See p. 461.)

The relations of the fimbria complex, including the commissura hippocampi, to the stria medullaris at this point are essen-

tially as described for Amblystoma (p. 424 ff.). Associated with the stria medullaris, are commissural fibers between the posterior poles of the hemispheres running by way of the commissura superior. This is the commissura pallii posterior.

On account of the absence of the diencephalic flexure in these brains the configuration of the thalamic structures bordering the telencephalon is very different from that of larval urodeles, but is the same in principle. The median and lateral ventral parts of the hemisphere pass back into the nucleus preopticus and prominentia fascicularis as before. The whole of the large nucleus preopticus, which belongs to the unevaginated forebrain or telencephalon medium, is overlapped dorsally by diencephalic structures, the sulcus diencephalicus ventralis forming the boundary between them (see figs. 33 to 38). As in the adult frog, this nucleus is very large and shows considerable internal differentiation. Correlated with the straightening out of the diencephalic flexure and the further evagination of the cerebral hemispheres in the anuran larvae we find, as mentioned above, that the eminentia thalami at the caudal border of the interventricular foramen is reduced to a small vestige, the nucleus of the hippocampal commissure (figs. 32 and 33).

Immediately caudal to the foramen the pars ventralis thalami enlarges to assume the same relations as in urodeles, being bounded by the sulcus medius and sulcus ventralis.

The ventro-lateral part is extended for a considerable distance behind the posterior pole of the hemisphere into the unevaginated telencephalon medium dorso-laterally of the preoptic nucleus (figs. 33, 34, 35). This tissue, including a part of the primordial corpus striatum, is in the course of further development almost all evaginated into the hemisphere proper, as shown by the adult configuration.

The habenula and superior commissure are placed far forward, as in urodeles, and the pars dorsalis thalami lies almost wholly caudal to these structures. The two segments of the sulcus diencephalicus dorsalis separate the epithalamus from the thalamus as described on p. 431 for urodeles.

The corpus geniculatum laterale appears near the rostral end

of the thalamus and accompanies the lateral border of the nucleus dorsalis thalami for its entire length. It also overlaps to some extent the pars ventralis thalami (figs. 36 to 39).

Gaupp in 1899 summarized the most important data upon the structure of the brain of the adult frog in the literature up to that date, together with a wealth of new observations, and his excellent account should be the point of departure for all subsequent work. I have examined a very large number of brains of different species of frogs prepared by diverse methods, including those of Weigert, Nissl, Golgi and the silver reduction method of Ramóny Cajal. The details of my observations must be reserved for later publication and we shall here consider only such general features as have a direct bearing upon the problems of the subdivision of the hemisphere and the morphological relations of these parts. The necessary figures will be found in the works of Gaupp ('99), Kupffer ('06), Kappers ('08), B. Haller ('08), Snessareff ('08) and the brothers Ramón y Cajal. See also fig. 40.

In the adult frog, as already stated, the fundamental parts of the hemisphere have attained the typical amphibian form. The shape of the hemisphere has considerably changed, as compared with urodeles and larval anurans, especially by the further evagination of structures found in earlier stages in the telencephalon medium, by the consequent contraction of the interventricular foramina and by the relative increase in the dorso-ventral diameter of the hemispheres. The lateral ventricles are dilated vertically and contracted laterally, thus giving to the cross-section of the hemisphere dorsal and ventral angles which form sharp boundaries between the lateral and medial parts. We shall next review the form and distinguishing characters of the five parts of the cerebral hemisphere of the frog.

The peculiar fusion of the olfactory bulbs seems to be confined to the bulbs themselves, as in the tadpole, though the participation of a small amount of secondary olfactory tissue (lobus olfactorius anterior) cannot certainly be excluded. Some fibers in diffuse formation cross the median plane in this region.

The pars ventro-medialis hemisphaerii (epistriatum, P. Ramón y Cajal; eminentia septalis, Gaupp; pars septalis hemisphaerii,

Kupffer) is included between the ventral angle of the hemisphere and the fissura limitans hippocampi. It is bounded rostrad by the olfactory bulb and caudad by the lamina terminalis. Within this part there is a highly differentiated cellular mass, the corpus precommissurale or nucleus medialis septi, which extends backward dorsally into the lamina terminalis and both below and above the interventricular foramen. These cells form the "bed" of the anterior and dorsal (or anterior pallial) commissures. The portion of the nucleus medianus septi which extends caudad above the foramen is very much enlarged in some species of frogs. It is well named by Kappers ('08) the pars fimbrialis septi, for its cells are in intimate relation with the fimbria fibers passing between the dorso-median and dorso-lateral parts and the ventro-median part which form one component of the complex termed tractus olfactorius septi.

B. Haller ('08, p. 373) designates this nucleus "Ammonskern" and considers it a part of the "Ammonswulst" or primordial hippocampus. He cites Edinger as teaching that the Ammonskern alone is the precursor of the hippocampus, but in the last edition of Edinger's Vorlesungen the pars dorso-medialis is designated cortex ('08, p. 306, fig. 276). The ventro-median part receives fibers from the olfactory bulb in front and is broadly connected with the hypothalamus through the median forebrain bundle, both by ascending and by descending tracts which partially decussate in the anterior commissure. It also has broad fibrous connections with the other parts of the hemisphere.

The pars ventro-lateralis is included between the ventral angle of the hemisphere and the fissura endorhinalis (sulcus limitans lateralis, Gaupp). It is bounded rostrad by the olfactory bulb; the caudal boundary is fixed dorsally by the so-called corpus striatum laterally of the lamina terminalis, while ventrally it passes over without interruption into the prominentia fascicularis (Gaupp) of the thalamus, which is a part of my pars ventralis thalami. In front it receives fibers from the olfactory bulb which for the most part come from the bulbulus accessorius and join to form a compact fascicle of unmedullated fibers which passes backward close to the ventricle and immediately ventral to the

zona limitans lateralis. These fibers have been variously interpreted by different authors. I have Golgi impregnations which show without ambiguity the whole course of this tract. Its fibers reach the so-called corpus striatum where they break up into a dense neuropil among the dendrites of the "striatum" cells. Fibers emerge from this neuropil and decussate in a separate (unmedullated) slip of the anterior commissure which connects the "striata." Others connect with the rostral end of the hypothalamus, where they decussate wholly or partially in the postoptic commissure system. The latter are probably both ascending and descending fibers, and the ascending fibers correspond to the tractus pallii of selachians, as has been pointed out to me by Professor Johnston. There are very numerous unmedullated fibers which leave the neuropil in the "striatum" to arborize freely in the adjacent lateral wall of the hemisphere and posterior pole. These come chiefly from the hypothalamic tract. Medullated fibers leave the nucleus for the pars ventralis thalami, some of which decussate in the anterior commissure. These form part of the tractus strio-thalamicus. Finally, there is the medullated and unmedullated connection with the stria medullaris which I interpret as tractus habenulo-striaticus (see p. 429). These relations are shown diagrammatically in fig. 41.

There is no differentiated corpus striatum in the Amphibia in the sense in which this term is used in mammals. The elements from which it is to be differentiated are present in the pars ventrolateralis.

The lateral forebrain tract is related to this part as in urodeles, its ascending fibers including strong tracts from the colliculus inferior and from the pars dorsalis thalami and corpus geniculatum laterale. These fibers terminate in both the ventral and dorsal lateral parts of the hemisphere and partially decussate in the anterior commissure.

The pars dorso-lateralis (formatio pallialis lateralis, Gaupp) lies between the fissura endo-rhinalis and the dorsal angle of the hemisphere. It extends from the olfactory bulb to the posterior pole, of which it forms the lateral wall. It receives a strong tract from the olfactory bulb and is broadly connected by very com-

plex tracts with the other three parts of the hemisphere. Its connections show clearly that it corresponds, at least in part, with the nucleus sphaericus of the reptiles and the lobus pyriformis of the lower mammals.

The pars dorso-medialis (eminentia pallialis medialis, Gaupp; septum, P. Ramón y Cajal; primordium hippocampi, Kappers). In the frog the whole of this part becomes primordium hippocampi but in some of the urodeles it seems probable that only its medial portion should be so designated. It extends the entire length of the hemisphere. In front of the lamina terminalis it is bounded ventrally by the fissura limitans hippocampi; behind this level it forms the whole median wall of the hemisphere. For its entire length it forms the thickest part of the wall.

At its rostral end the primordium hippocampi is enlarged and here it receives secondary olfactory fibers from the olfactory bulb, and is connected by a great mass of unmedullated ascending and descending fibers with the underlying nuclei of the septum. All portions of the primordium are connected by strong association tracts with the dorso-lateral part of the hemisphere. The fibers of the commissura pallii anterior arise throughout its mass and P. Ramón y Cajal ('05, fig. 4) figures a Golgi impregnation of this commissure which shows that the free terminal arborizations of its fibers after decussation also reach the lateral wall of the hemisphere directly.

The cells of the primordium hippocampi are arranged diffusely, being scarcely more dense next to the ventricle than elsewhere; but there is no true cortex developed except perhaps at the dorso-median angle of the hemisphere. P. Ramón y Cajal ('05, p. 185 and fig. 7) describes here a superficial layer of tangential cells which have well differentiated axons leaving the cell body or the base of one of the thick dendrites and passing ventrally along the medial superficies of the hemisphere. I have impregnations of these cells in Golgi preparations of Rana pipiens which show that in the rostral half of the hemisphere they extend from the dorsal angle ventrally along the entire medial surface of the primordium hippocampi instead of being limited to the dorsal angle as figured by Ramón y Cajal. Medullated fibers which I consider to belong

to the columna fornicis (see p. 423) pass downward into the septum from amongst these cells, but I have not been able to establish their connection with them. If this connection exists, then these cells constitute a true cortex hippocampi. The superficial layer of tangential cells can be easily distinguished from the deeper cells in sections stained with toluidin blue on account of their difference in form, which is shown diagrammatically in fig. 40. There is, however, no cell-free medullary or molecular layer between these cells and the so-called pyramidal cells which fill the remainder of the primordium.

In addition to the true columna fornicis fibers which pass down through the septum and lamina terminalis in the typical vertebrate way, as described above, there are other connections which are functionally of the same type. Unmedullated fibers from all parts of the primordium hippocampi and from the dorso-lateral part (the latter curving around the dorsal and mesial surface of the hemisphere) pass downward into the nucelus medianus septi. Some of these form one component of the complex tract known as the tractus olfactorius septi and may pass through this nucleus to enter the hypothalamus by the way of median forebrain tract, in which case they should be associated with the medullated tract already described as columna fornicis. Others certainly end within the nucleus medianus. Since the latter sends its fibers to the hypothalamus, this path with its interpolated synapse may also serve functionally the same purpose as the columna fornicis. No fibers pass from the true columna fornicis into the stria medullaris, as in reptiles and mammals, to form the tractus cortico-habenularis; but there are other connections which functionally belong to the fornix system.

Some of the characteristic relations of the rostral part of the hemisphere are shown in the diagrammatic cross section, fig. 40.

In no Amphibia do the fibers of the commissura hippocampi system take the course characteristic of the reptiles and mammals, viz., above the interventricular foramen, to cross in the lamina terminalis in front of the foramen; on the other hand, they cross behind the foramen by two paths. One of these is ventral in the

lamina terminalis, the other is dorsal in the commissura superior. The configuration is such that the primordium hippocampi touches the anterior border of the thalamus and, as in the larva, these two commissural tracts converge at the junction of the taenia fornicis with the taenia thalami.

The relations here are similar to those of urodeles and frog tadpoles (see pp. 426 and 439), except for the great increase in size of the supraforaminal part of the precommissural body, or pars fimbrialis septi, and the reduction of the eminentia thalami to form the small but dense nucleus of the hippocampal commissure, which lies at the junction of the fimbria fiber complex with the stria medullaris ventrally of the pars fimbrialis septi. The summary of fiber connections at this point given for urodeles on p. 427 applies also to the frog.

The posterior pole of the hemisphere is that portion of the two dorsal parts which projects caudad beyond the level of the lamina terminalis; it is the parathalamic brain. Here the dorso-lateral and dorso-medial parts come together as in urodeles and in some measure lose their distinctive characteristics, suggesting the undifferentiated condition of the so-called lobus hippocampi or nucleus pyriformis (Sheldon) of teleosts (see p. 451). It receives numerous secondary olfactory fibers from the olfactory bulb and gives rise to the tractus cortico-habenularis lateralis (tractus taeniae, Edinger) and the commissura pallii posterior, as has been already suggested by Elliot Smith ('03, p. 495).

The hemispheres of the adult frog are so much farther evaginated, as compared with the larvae and the urodeles, as to make correlation with reptiles and higher forms relatively easy. The dorsal parts are clearly separated from the ventral parts for the entire length of the hemisphere both by the external and internal zona limitans and by total fissures; and the ventral parts alone are in direct massive contact with the corresponding parts of the diencephalon. The dorso-median part is in contact with the ventral part of the thalamus, but not with any part of the thalamus dorsally of the sulcus diencephalicus medius. This contact is probably secondarily acquired in Amphibia (see p. 420) and it does not occur in Amniota.

The relations of the ventro-median part remain practically unchanged except for further growth beyond the lamina terminalis.

The ventro-lateral part is as directly continuous with the pars ventralis (prominentia fascicularis) of the thalamus as in the larva. The elements of the primordial corpus striatum are more completely evaginated than in the larva; nevertheless the caudal end of this complex still remains behind the interventricular foramen in intimate relation with the lateral forebrain tract in the unevaginated wall in the same morphological level as the eminentia thalami of the larva.

The pars dorsalis thalami is greatly enlarged in the frog and its whole lateral surface is differentiated as corpus geniculatum laterale to receive collaterals and terminals of the optic tract. The fiber connections of the thalamus are essentially as already described for the urodeles (pp. 433, ff.), save for a much larger proportion of optic projection fibers from the corpus geniculatum laterale in the lateral forebrain tract, and also a larger component from the midbrain.

Gaupp ('99, p. 80) gives a very clear analysis of the walls of the diencephalon. He divides the central grey of the thalamus into habenular ganglion and superior, medial and inferior parts. The last belongs in the hypothalamus and the superior and medial parts correspond with the pars dorsalis and pars ventralis thalami of my description. The sulcus diencephalicus dorsalis is present and also the sulcus medius. Gaupp does not name these sulci, but gives to the cell-free zone bordering the sulcus medius the name zona limitans superior. The sulcus diencephalicus inferior is absent, but its position is marked by a similar "zona limitans inferior." Gaupp correctly describes the pars dorsalis (superior) thalami as ending in attenuated form rostrally of the habenula, and the pars ventralis (his medialis) as extending forward into the nucleus supracommissuralis, or nucleus of the hippocampal commissure.

COMPARISONS WITH FISHES

The brief account of the brain of Lepidosiren recently published by Elliot Smith ('08) shows striking resemblances with the Amphibia, with, however, divergent lines of specialization. The differentiation of a layer of cortical cells in the dorsal region is reptilian in type, though these cells are probably represented in the frog also (see fig. 40), and the ventral enlargement of the tuberculum olfactorium is unique. The similarity to the amphibian conditions is, in fact, so close as to justify us in reviewing Elliot Smith's conclusions in the light of the preceding discussion.

He describes the corpus paraterminale as comprising practically the whole median wall dorsally of the tuberculum olfactorium and further states ('08, p. 533) that it is divided into dorsal and ventral parts by an "indentation of epithelial cells" which the figures show to mark the level of the interventricular foramen. The ventral part is clearly the nucleus medianus septi. It is equally clear that the dorsal part is chiefly composed of the equivalent of the amphibian pars dorso-medialis, or primordium hippocampi. In the adult frog Elliot Smith has recognized ('03, p. 497) the distinction between the primordium hippocampi and that portion of the paraterminal body which lies dorsally of the interventricular foramen (pars fimbrialis septi of Kappers); but he has failed to make the corresponding analysis here. An examination of the photographs reproduced in his paper suggests that the resemblance to the Amphibia is even closer than Elliot Smith seems to have recognized.

The arcuate fissure is absent in Lepidosiren, as in the Amphibia. The fissure *B* of Elliot Smith's fig. 1 would appear at first to be the fissura limitans hippocampi, which marks the position of the zona limitans in the frog; but this I think is not the case. It is more nearly comparable with the ependymal groove which is seen in the frog larva (fig. 30), and marks the level of the dorsal boundary of the interventricular foramen. In both Lepidosiren and the frog tadpole the precommissural body (nucleus medianus septi) extends dorsally of this groove, while the fissura limitans hippocampi always lies wholly dorsal to the precommissural body. The

photographs (figs. 2 and 14 of Elliot Smith's paper) show clearly above this fissure *B* a denser mass of cells separated from the overlying pars dorso-medialis by a zona limitans. These cells mark the most dorsal limit of the nucleus medianus septi or precommissural body, as I have defined it in this paper, and seem to be exactly comparable with the larger mass of cells seen in larval and adult frogs in the corresponding position. They may extend caudad for a short distance above the interventricular foramen, though the figures suggest that this is improbable.

Elliot Smith explains briefly the morphology of the rudimentary pallial formation of Lepidosiren and I shall return to the consideration of his valuable suggestions in the final discussion (p. 490). None of the available descriptions of the brain of Ceratodus are well adapted for the comparisons here instituted. Bing and Burckhardt ('05, fig. 15, p. 550) give a figure of the embryo which shows that at this stage the cross section of the forebrain at the level of the interventricular foramen is almost identical with that of the just hatched Necturus (cf. Kingsbury, '95, pl. ix, fig. 8). Protopterus, as figured by Burckhardt ('92) is much more similar to Lepidosiren than is Ceratodus.

The fiber tracts of the Dipnoi are not sufficiently well known to permit us to control all of the homologies here suggested; but so far as known they support them. Burckhardt's meager account of the forebrain tracts of Protopterus shows that the commissura pallii anterior is present (termed corpus callosum), but it is not clear whether it enters the lamina terminalis behind the interventricular foramina as in Amphibia or in front of them as in elasmobranchs. His figures of cross-sections show that the diencephalon and telencephalon are divided longitudinally into parts which correspond closely with those of Lepidosiren and Amphibia. He discusses (p. 25) these longitudinal zones in comparison with those of urodeles, but since he incorrectly homologized all of the parts of the hemisphere (see Elliot Smith, '08), his analysis is not fruitful.

The cerebral hemispheres of the higher ganoids and teleosts have developed in a different direction from that taken by the Amphibia. In the olfactory bulb alone is the massive wall of

the telencephalon fully evaginated. The telencephalon medium is elongated and in its massive floor and side walls are differentiated structures corresponding with the four parts into which we have divided the amphibian hemispheres, the membranous roof of the ventriculus impar being the equivalent of the median and lateral plexuses of the urodele hemispheres. In the higher fishes the enlargement of the massive parts has led to their lateral eversion, not to an evagination of the whole wall as in Amphibia. The subdivision of the teleostean "hemisphere," therefore, resembles more closely the plan of the diencephalon than that of the telencephalon of Amphibia (see p. 477 and fig. 83), but is in important respects unlike both of them.

This fundamental difference between the teleostean and amphibian types of cerebral hemispheres was first pointed out by Mrs. S. P. Gage ('93) and the idea has since been elaborated by Kappers and Edinger. An analysis of the teleostean forebrain will shortly be published by Dr. R. E. Sheldon, in connection with which this problem is fully discussed. Accordingly, no further reference will be made to it here save to add that, though very different morphogenic factors have operated in the evolution of the teleostean brain as compared with elasmobranchs and amphibians, nevertheless the homologous parts are recognizable. Our conclusions follow, with some important modification in details, the interpretation of Johnston ('10, p. 155).

The selachians have been recently re-examined by Sterzi and from his descriptions it is evident that in these brains the telencephalon medium is extensive. In the lower elasmobranchs the evaginated part contains little except the olfactory bulbs, and even in the higher sharks the remaining parts of the telencephalon are differentiated for the most part as local thickenings of the wall, with a relatively small amount of evagination as compared with the Dipnoi and Amphibia. In the diencephalon of Acanthias one of Sterzi's figures ('09, p. 577, fig. 232) shows the sulci arranged in much the same way as in urodeles. See also Burckhardt's figure of Scymnus ('07, p. 354, fig. 21). These probably separate homologous parts in amphibians and elasmobranchs, but the determination of this point must await a fuller study of their

fibrous connections. Further consideration of these brains will be reserved pending the appearance of a paper on the subject now in press by Professor Johnston.

Some considerations regarding the brains of cyclostomes will be found in the discussion on page 470.

REPTILIA

The reptilian cerebral hemisphere is characterized chiefly by the highly developed corpus striatum complex and by the presence of true cortex in the pallium. This cortex is arranged in three distinct laminae (cf. fig. 61), the lateral cortex (palaeocortex of Kappers, '08), the dorsal cortex (cortex ammonis, Kappers) and the dorso-medial cortex (fascia dentata, Kappers). The dorsal and dorso-medial cortex both extend to the extreme rostral end of the hemisphere, the latter here resting directly upon the underlying secondary olfactory center of the olfactory crus. This condition is repeated exactly in the lower mammals in relations which permit us to homologize the dorso-medial cortex of the lizard without question as cortex hippocampi. Comparison with the adult frog shows that there too the dorso-medial part of the hemisphere extends forward in the same way to the olfactory bulb over the pars ventro-medialis, a fact which justifies us in calling the pars dorso-medialis of the frog primordium hippocampi for its entire length.

The reptilian septum is further differentiated as compared with the Amphibia, and is in fact similar to that of lower mammals. The unitary collection of cells in its dorsal part which in Amphibia was termed nucleus medianus septi, or precommissural body, is here subdivided into several nuclei. The term precommissural body may be retained for all of these nuclei related to the amphibian body of the same name, including the reptilian bed nuclei for the commissures in the lamina terminalis (and the nucleus of the commissura pallii posterior of lizards), though it must be borne in mind that the term is used here in a more restricted sense than by Elliot Smith. That author's pre-commissural or paraterminal body of reptiles includes also the vestigial primor-

dium hippocampi, which, as will appear immediately, I exclude from it. The nuclei of the hippocampal commissure and tractus cortico-habenularis (see p. 461) should also be excluded.

The most highly differentiated cortex of reptiles is the dorso-medial. Its relations to the ventro-medial part of the hemisphere have been investigated in adult brains of several species of reptiles and in the extensive series of embryos in the Harvard Embryological Collection.

Chelonia. We shall consider first the turtles. In transverse sections of an embryo of Chrysemys marginata of 16.7 mm. (Harvard Embryological Collection, no. 1092) the ventro-medial part, or septum, has already attained great size; and, although the process of proliferation of neuroblasts from the central grey is still active, the principal regions of the adult hemisphere can be recognized. The dorso-medial cortex is well defined; the lateral cortex less clearly. A section through the middle of the septum (fig. 43) shows that the medial wall of the hemisphere is subdivided, as in Amphibia, by a definite zona limitans into dorsal (hippocampal) and ventral (septal) parts; but the lateral wall does not conform to the amphibian type. The tuberculum olfactorium, precommissural body (nucleus medianus septi) and nucleus accumbens septi can be recognized. The locus of the nucleus lateralis septi lies between the two last. Fibers of the fornix and commissura hippocampi systems are seen crossing the zona limitans. A section taken 150 micra farther caudad (fig. 44) shows dorsal to the zona limitans a thin portion of the wall which contains fimbria fibers and a few nuclei. In a section taken 50 micra farther back and immediately in front of the interventricular foramen (fig. 45) an extensive septum ependymale appears and above it a slight thickening containing the fimbria fibers, the limbus medullaris of His. This belongs to the dorso-median part of the hemisphere and represents a small residue of the unspecialized amphibian primordium hippocampi, the remainder of the primordium having been transformed into cortex hippocampi (dorso-medial cortex). The commissura hippocampi crosses in the lamina terminalis in the plane of this section, its fibers having curved downward rostral to the interventricular foramina and septum ependymale.

In Chrysemys marginata of 27 mm., cross sections show that the medial and lateral nuclei of the septum are well differentiated and there is a membranous sac or pocket which projects forward on each side above the lamina terminalis and its commissures (fig. 46). This is the recessus superior which Elliot Smith ('03) has recognized in monotremes and other vertebrates and which Mrs. Gage has recently demonstrated in a five weeks human embryo (at the Boston meeting of the Association of American Anatomists, Dec. 28, 1909). The paraphyseal tubules spring from its dorsal surface and the lateral plexuses arise immediately behind it. The recessus superior has been described in the gecko embryo by Tandler and Cantor ('07).

I have preparations of adult brains of Chrysemys marginata by the Weigert method which show that here also there is an undifferentiated region between the precommissural body and the cortex hippocampi which contains fimbria fibers and scattered small cells. This is a vestige of the unspecialized primordium hippocampi which has not assumed cortical characters. In the middle region of the septum the nucleus lateralis septi has grown up dorsally into contact with the cortex hippocampi on its ventricular side very much as shown for the alligator in fig. 66; but both rostral and caudal to this level the primordium hippocampi occupies the whole thickness of the wall, where it is bounded below by the fissura limitans hippocampi. It is everywhere separated from the cortex hippocampi by the fissura arcuata.

In the adult box tortoise, Cistudo carolina, the same conditions prevail save that the lateral nucleus of the septum is smaller here. Fig. 47 illustrates the relations immediately rostral to the interventricular foramen in Cistudo.

In none of the turtles which I have examined, either embryonic or adult, do any cells of the precommissural body extend backward above the interventricular foramina, as in the frog and lizard.

The relations of the telencephalon to the diencephalon are clearly illustrated by figs. 48 and 49, where the resemblance to urodele larvae is very striking. These sections of a 12.3 mm. embryo of Chrysemys marginata are cut transversely to the sagittal plane and so inclined that the dorsal surface is much farther

caudad than the ventral. Fig. 48 passes through the caudal border of the interventricular foramen on the left side and immediately behind it on the right. It passes through the anterior commissure below and the dorsal sac rostral to the habenulae above; cf. fig. 50, a sagittal section of an older embryo. From the interventricular foramen, which is very wide immediately rostral and dorsal to this level, two ependymal grooves extend backward in the same relations as in urodeles, the sulcus diencephalicus medius above and the sulcus diencephalicus ventralis below. Between these is the pars ventralis thalami which is seen to connect broadly with the lateral wall of the hemisphere. In fact it is the precise equivalent of the eminentia thalami of amphibian larvae. It is completely separated from the dorsomedian part of the hemisphere by the membranous posterior chorioidal fold and incompletely separated from the ventromedian part by the sulcus ventralis. In the adult its rostral end is carried forward, as in the frog, to form the nuclei of the hippocampal commissure and tractus cortico-habenularis (see p. 461).

Dorsal to the sulcus medius is the pars dorsalis thalami, and above this the sulcus dorsalis and habenula. These relations come out still more clearly in a section taken a little farther back (fig. 49).

Fig. 50 is a sagittal section through the brain of an older embryo of Chrysemys marginata 26.7 mm. long and fig. 51 a parasagittal section from the same embryo illustrating the relations of the diencephalic sulci. The pars dorsalis thalami is here, as in reptiles and mammals generally, divided into a central major part and a nucleus dorsalis containing more densely crowded cells. Both embryological and phylogenetic development show that these two parts have a common origin.

The subdivisions of the diencephalon are shown with great clearness in the models of the brains of gecko embryos figured by Tandler and Cantor ('07, see especially figs. 10, 14, and 17). The boundary between the pars ventralis thalami and the hypothalamus in these embryos is less distinct than in the Amphibia and the chelonian embryos here described. Tandler and Cantor's

scheme of subdivision of the reptilian prosecephalon seems to me faulty, as will appear beyond. This is probably in part due to the fact that they did not study sufficiently early stages but chiefly because of imperfect knowledge of the functional factors involved in development such as would be brought out only by a study of the adult fiber tracts.

Lacertilia.—In the lizards the precommissural body is much more highly developed than in the turtles and this leads to important differences in the development of the septal region. In the adult the nuclei of the septum extend much farther dorsad and caudad than in turtles in relations similar to those of the pars fimbrialis septi of the frog.

The Harvard series 1601 comprises frontal sections of an embryo of Lacerta of 7.6 mm. (measured as coiled, total length unknown) cut in approximately the same plane as the older embryo shown in figs 53 to 57, though somewhat more nearly transverse to the long axis of the diencephalon. At this age the commissura pallii anterior is present, though small, but the commissura pallii posterior has not yet appeared. Tandler and Cantor ('07) find that in the gecko also the commissura pallii anterior develops earlier than the commissura pallii posterior.

A section through the mid-region of the septum, corresponding approximately to the plane of fig. 44 of Chrysemys, shows that the nucleus lateralis septi has grown dorsad so as to invade the ependymal border of the primordium hippocampi (fig. 42), a condition which is found in this region of adult Chrysemys but not in the embryo of the age shown in fig. 44. This position of the lateral nucleus is characteristic of the entire length of the septum of Lacerta at this and all later ages. The ventral limit of the original primordium hippocampi can, however, generally be recognized on the outer surface of the hemisphere of adult lizards. The region corresponding with the membranous septum ependymale of of the Chrysemys embryo of 16.7 mm. (fig. 45) is massive in the Lacerta embryo of 7.6 mm., containing fimbria fibers and cells of the medial and lateral nuclei of the septum.

Passing caudad in this series of sections of Lacerta, at the level of the commissura pallii anterior a portion of the nucleus medianus

septi extends dorsally of the commissure, giving relations very similar to those of the older embryo of fig. 54. The nucleus lateralis septi also extends for a very short distance dorsally of the interventricular foramen, but not so far as in the older embryo, figs. 55 and 56. No cells from the septum extend farther backward than the dorsal border of the foramen, the nucleus of the commissura pallii posterior being as yet undeveloped, in correlation with the absence of the commissure itself.

Figs. 52 to 57 illustrate sections through the brains of older embryos of Lacerta (about 36 mm. long when uncoiled, head 5 mm. long) from the Harvard Collection. Fig. 52 is a parasagittal section through the brain of one of these specimens and upon it is indicated the approximate plane of section of each of five sections through another specimen. In this figure the precommissural body is seen to rise up in front of the interventricular foramen and extend backward for a short distance above it. The nucleus lateralis septi, separated from the nucleus medialis by fimbria fibers, extends far dorsal and caudad into contact with the margin of the cortex. The thalamus is related to the telencephalon as in the turtles already described.

Figs. 53 to 57 illustrate a series of sections taken in the planes indicated on fig. 52. The primordium hippocampi is clearly defined on the medial surface of the brain, being bounded by definite sulci at the level of fig. 54. The fissura limitans hippocampi separates the precommissural body from the primordium hippocampi, and the latter is separated from the dorso-medial cortex (cortex hippocampi) by the fissura arcuata. Attention is especially called to the fact that the fissura arcuata lies within the hippocampal formation, not below or above it, and to the importance of this fact in determining its homology in vertebrates.

The fibers of the commissura hippocampi are here divided, one part crossing in the lamina terminalis as in turtles, here termed the commissura pallii anterior (figs. 53 and 54), and another part derived from the posterior pole crossing in the velum transversum at the rostral border of the epithalamus (figs. 56 and 57).

A special collection of cells belonging to the supra-foraminal extension of the nucleus lateralis septi passes backward and far

lateralward along those fibers of the fimbria which enter the commissura pallii posterior (figs. 56 and 57). This nucleus I term the nucleus of the commissura pallii posterior. It clearly is a part of the precommissural body. Thus the commissura pallii posterior, like all of the commissures in the lamina terminalis, is embedded in a matrix of cells belonging to the precommissural body, though in this case these cells lie far laterally and not in the velum transversum itself.

The relations of the commissura pallii posterior to the precommissural body were first described by Elliot Smith ('03) in Sphenodon and lizards. Two embryos of Sphenodon in the Harvard Collection permit a re-examination and full confirmation of his description. Figs. 58 and 59 pass horizontally through the crossing of the commissura pallii posterior in the velum transversum in an embryo of 25.2 mm. Fig. 58 shows the nucleus of the commissure at its greatest extent and lying much nearer the midline than in Lacerta. Fig. 59 is 70 micra farther dorsal and illustrates its farthest extension into the fimbria. The relation of the nucleus to the precommissural body is broader and more evident than in Lacerta and similar in plan.

Kupffer ('06) has described the brains of advanced embryos of Anguis fragilis in which the structure of the septum is clearly brought out. In fig. 60 I present a copy of his drawing of a section through the lamina terminalis. The precommissural body is very large and is termed eminentia medialis. At the level figured it is divided into a ventral part forming the commissure bed (*tr.*), a lateral part (*em.*), the nucleus lateralis septi, and a dorsal mass (*m'*). These relations conform very closely to those of the lizard embryo (fig. 54). Kupffer's nucleus, *m*, which he regards as part of the septum and identifies with Gaupp's ganglion septi of the frog, is the primordium hippocampi and his fissure (*f.c.h.*), is the fissura limitans hippocampi of my description. A section taken through the commissura pallii posterior shows an "unbekannter Kern" (Kupffer's fig. 259, *k*) which is clearly the nucleus of this commissure, as described above for Lacerta.

Figs. 61 and 62 illustrate transverse sections through the brain of an adult lizard, Phrynosoma, the so-called horned toad of the

western United States. In this animal the nucleus lateralis septi is smaller than in many other lizards.

In the lamina terminalis (fig. 61) the precommissural body has risen above the preoptic recess and forms the "bed" of the commissures. It is broken up by the fiber tracts of the anterior commissure, commissura pallii anterior and fornix into detached clusters of cells, one which (*n.c.p.*), extends caudad above the interventricular foramen as in larval Lacerta. The section figured is slightly oblique, the left side being farther caudad and showing the relations immediately rostral to the foramen. The relations of this nucleus to the commissura pallii posterior caudad of the foramen are seen in fig. 62. Dorsally of these parts of the precommissural body is a small residue of the primordium hippocampi, filled with fibers of the fimbria. The fissura arcuata is incomplete, not being marked on the ventricular surface, this region being invaded by the nucleus lateralis septi. The cortex of the dorso-medial wall (cortex hippocampi) is much more highly developed, with more numerous and more densely crowded cells, than is any other part of the cortex. Fig. 62 is taken through the foramen interventriculare on the right side and the commissura pallii posterior just behind the foramen on the left. On each side is seen a small remnant of both the precommissural body (*n.c.p.*) and the primordium hippocampi.

Figs. 63, 64 and 65 illustrate horizontal sections through the brain of another lizard, Sceloporus. The sections are slightly oblique, the left side being a little farther dorsal than the right. The left side of fig. 63 shows the dorso-median cortex at its greatest extent, a condition which prevails at all levels dorsally of the one figured. Immediately ventrally of this level, as shown by the right side of fig. 63, the caudal wall of the hemisphere becomes membranous and the thick primordium hippocampi appears in the median wall. Fig. 64 passes through the interventricular foramen on the right side and shows more densely crowded masses of cells surrounding the foramen; these belong to the precommissural body. The left side shows that this nucleus extends above the foramen also as nucleus of the commissura pallii posterior (*n.c.p.*). Rostrally of the foramen is the thick septum, which is

bounded farther forward by the perfectly differentiated dorsomedial cortex. A remnant of this cortex is also seen at the caudolateral angle of the hemisphere. Similar conditions prevail ventrally of the foramen (fig. 65), save that here the precommissural body occupies a much larger part of the "septum," as well as the "bed" of the commissura pallii anterior.

Laterally of the median plane the commissura pallii posterior lies in contact with the stria medullaris for a short distance at the rostral border of the pars dorsalis thalami and habenula (figs. 52, 64), and some of my preparations of Phrynosoma and Sceloporus seem to show a fibrous interchange between these tracts. But my specimens of these lizards are not well adapted for the study of fiber tracts and the nature of these fibers must be left undecided. Most of the fibers of the stria medullaris pass farther caudad to reach the habenula and superior commissure and do not share in this contact with the commissural fibers.

Many years ago Kupffer ('93, p. 57) commented upon the fact that in the Amphibia the superior commissure lies farther rostral as compared with the other elements in the diencephalic roof than in most other vertebrates, and in his last work ('06) he figures the relations of these elements in a large series of vertebrate brains. In Amphibia the relations of the fibers of the commissura pallii posterior have perhaps determined the position of the superior commissure and in lizards we seem to have an intermediate condition. In all higher forms there is no direct connection of any sort between the posterior poles of the hemispheres and the diencephalon, probably by reason of an increase in the extent of the posterior chorioidal fold and fissura chorioidea.

Crocodilia.—The brain is more highly differentiated in the Crocodilia than in other reptiles; and I shall describe briefly some of the features of the brain of a young specimen of Alligator mississippiensis 25 cm. long, stained by the silver reduction method of Cajal.

In the mid-region of the septum (fig. 66) the nucleus lateralis is seen to receive fibers from the columna fornicis and commissura hippocampi as described by Cajal ('04, p. 1055) in the mouse. The wide fissura arcuata extends far rostrad, as in lower mammals,

and its floor is occupied by an undifferentiated primordium hippocampi, filled with fimbria fibers and containing scattered large cells. Comparison with embryonic and adult turtles indicates that the position of the nucleus lateralis septi laterally of the primordium hippocampi is secondarily acquired. The nucleus lateralis arises wholly ventral to the primordium hippocampi and grows upward into its present position late in the ontogeny.

The columna fornicis passes downward rostral to the anterior commissure to enter the medial forebrain tract for the most part farther forward than the plane of this section. The tract designated fornix (*c.f.*) here contains also external arcuate fibers, *i.e.*, axons of the cells of the nucleus lateralis septi.

Fig. 67 passes through the rostral border of the anterior and hippocampal commissures and the caudal end of the nucleus lateralis septi, above which lies the primordium hippocampi containing scattered cells and fimbria fibers. Associated with the commissura hippocampi is a nucleus of densely crowded cells, which is clearly homologous with the nucleus of the commissura hippocampi described for Amphibia. Here it lies ventrally of the commissural fibers and just caudad of the level figured its cells cross the median plane. Cells of the precommissural body surround this commissure, the anterior commissure and the fornix and are mingled amongst their fibers, but this nucleus is sharply circumscribed and easily distinguishable from the precommissural body.

At this level the precommissural body is bounded above by the recessus superior and below by the preoptic nucleus. It disappears immediately caudad and there is no pars fimbrialis extending backward above the interventricular foramina. The tract marked fornix (*c.f.*) in this figure contains external arcuate fibers, columna fornicis and tractus cortico-habenularis.

At the level of the foramina (fig. 68) the nucleus preopticus is greatly enlarged and large fiber tracts pass between it and the corpus striatum complex.

The nucleus of the hippocampal commissure is in contact laterally with a similar nucleus among the fibers of the tractus cortico-habenularis as they pass upward and laterally from the fornix

toward the stria medullaris. I shall term the latter the nucleus of the tractus cortico-habenularis. These two nuclei have been differentiated from the single commissural nucleus of Amphibia, and they represent here also the rostral end of the pars ventralis thalami (cf. fig. 69). Fibers (probably collaterals) from the commissura hippocampi and tractus cortico-habenularis end freely among their cells. Other fibers from the same sources pass through the nuclei to enter the stria medullaris and still others pass between the nucleus of the tractus cortico-habenularis and the corpus striatum complex. The significance of the latter fibers is uncertain. Both nuclei, like their precursor in the Amphibia, clearly represent a correllation center of some sort interpolated in the cortico-habenular path, probably for efferent discharge into the somatic motor centers.

The nuclei of the septum, including the bed nuclei of the anterior commissure and commissura pallii anterior and posterior, belong morphologically in the pars ventro-medialis of the hemisphere and constitute a correlation center interpolated between the olfactory bulb and the cortex hippocampi on the one hand and the hypothalamus on the other hand. In amphibians, reptiles and mammals columna fornicis fibers (or collaterals) end in this nucleus, which is also related by association tracts with the corpus striatum complex in the ventro-lateral part of the hemisphere. Its chief path of efferent discharge is into the hypothalamus. (For the mammalian relations see Ramón y Cajal, '04, p. 1054).

In reptiles the tractus cortico-habenularis arises from the columna fornicis and commissura pallii anterior within the precommissural body, with whose cells it has a collateral connection, then passes by way of the stria medullaris to the habenula where correlation is effected with the somatic sensory centers of the pars dorsalis thalami before the discharge into the ventral tracts of the interpeduncular region.

The nuclei of the hippocampal commissure and tractus cortico-habenularis have still a different significance, having been differentiated from the rostral end of the pars ventralis thalami. They receive collaterals from the tracts to which they are related and are connected laterally with the striatum complex. In brief,

they provide for a correllation of the efferent impulses from the cortex hippocampi with the somatic motor correllation centers and for discharge through the latter.

From the preoptic nucleus at the level of fig. 69 the habenular tract arises in two parts, as in Amphibia, the tractus olfacto-habenularis medialis passing up internal to the basal forebrain bundle and the tractus olfacto-habenularis lateralis, laterally of this bundle. The rostral end of the pars dorsalis thalami has appeared and the limits of the pars ventralis are marked by sulci, as in the Amphibia.

I present one more illustration from this series (fig. 70), taken through the optic chiasma and rostral end of the massa intermedia, to illustrate the subdivision of the diencephalon. A nucleus dorsalis is differentiated as in lower mammals and it is separated from the remainder of the pars dorsalis by a shallow sulcus. The lateral part of the pars dorsalis is closely related with the corpus geniculatum laterale, as in the frog, and the optic radiations for the hemisphere go out in company with the tractus thalamo-corticalis.

Elliot Smith ('03 and '10) has given a critical review of the older ideas regarding the morphology of the reptilian cerebral cortex which it is unnecessary to summarize here. The precise homologies of the reptilian cerebral cortex in general cannot yet be stated, in the absence of sufficiently exact knowledge of the fiber connections of the various areas. The connections of the dorso-median cortex bordering the primordium hippocampi show it to be clearly cortex hippocampi; but how far laterally this formation extends we are not now in a position to decide. The nucleus sphaericus (of Adolf Meyer and Edinger, occipito-basal lobe of C. L. Herrick, epistriatum of Edinger and Kappers) in reptiles may now be compared, in a general way, at least, on the basis of its fiber connections, with the pars dorso-lateralis of the frog brain and the pyriform lobe and amygdala of the lower mammals; and it is very probable that the neopallium arose immediately dorsally of this center which is known to receive both olfactory fibers and projection fibers from lower segments of the brain. See the further discussion on p. 488.

The subcortical centers in the median wall of the reptilian hemisphere can be more precisely analyzed. The "septum" of earlier authors (intraventricular lobe of C. L. Herrick, paraterminal body of Elliot Smith, area precommissuralis septi of Kappers, eminentia medialis hemisphaerii of Kupffer) is of two-fold origin: (1) The larger antero-ventral component is a derivative of the primitive ventro-median area. The differentiated cellular mass in the dorso-caudal part of this ventral component is comparable with the nucleus medianus septi of the Amphibia, and to this mass I have applied Elliot Smith's name precommissural body. (2) The small remainder of the "septum" is a vestige of the unspecialized primordium hippocampi of the Amphibia. It is of dorso-medial origin and is morphologically of quite different type from the precommissural body.

The fissura arcuata has given rise to much controversy in mammalian neurology and in reptiles its morphology is equally difficult. In mammals the fissura hippocampi is a total fold of the median wall of the hemisphere and His has clearly shown ('04) that the fissura arcuata of the foetal brains which he has described is the precursor of this fissure. In mammals the hippocampus, or cornu Ammonis, is the part of the cortex which is folded into the ventricle by this fissure. In embryonic and adult reptiles (figs. 47, 54, 60, 66, 67) we have a similar fold of the median wall of the hemisphere which is commonly called fissura arcuata. The cortex hippocampi sometimes extends downward into the dorsal lip of this fissure, but never in reptiles into the ventral lip which is always formed by an unspecialized vestige of the primordium hippocampi containing fimbria fibers and sparse cells. Since the reptilian cortex hippocampi above this fissure was unquestionably derived from the dorsal part of the amphibian primordium hippocampi and since that portion of the reptilian primordium hippocampi which forms the ventral wall of this fissure is in like manner destined in mammals to be transformed into cortex hippocampi, I think it is clear that the fissura arcuata of the lacertilian embryo occupies the same relative position in the wall of the cerebral hemisphere as that of the mammalian embryo. Of course it does not necessarily follow that the fissures are homologous,

though I incline to believe that they are so, and therefore in reptiles I retain the name fissura arcuata, for I think it marks the position of the embryonic fissura arcuata and also that of the mammalian fissura hippocampi. Elliot Smith ('03, p. 469) in discussing the fissura arcuata of foetal monotremes, calls attention to a slight sulcus, β, between the fascia dentata and the paraterminal body (see fig. 71, where I present a copy of his figure of foetal Echidna), which he calls the sulcus limitans hippocampi, and he adds, "most writers on the reptilian brain regard the sulcus β as the homologue of the *Bogenfurche* (δ); this drawing shows how erroneous such a contention is." As a matter of fact, "most writers on the reptilian brain" have not distinguished at all between the two fissures marked δ (*Bogenfurche* or fissura arcuata) and β (sulcus limitans hippocampi) and my purpose in calling attention to the matter here is to do so. In adult reptiles the two fissures are sometimes distinct and the lips of the fissura arcuata are bounded above by cortex hippocampi and below by the undifferentiated primordium hippocampi. In other cases one or the other may be wanting.

Of these the fissura limitans is phylogenetically the older, for it is homologous with the fissure which I have so named in the Amphibia, while the true fissura arcuata is not found below the reptiles. (The so-called fissura arcuata of the frog is the fissura limitans, for it lies entirely ventral to the primordium hippocampi, while the fissura arcuata always lies *within* the hippocampal formation.) It is true, as implied by Elliot Smith's remark quoted above, that the fissura arcuata of the adult reptile and the sulcus limitans of the foetal Echidna both mark the ventral limit of the differentiated cortex of the hippocampal formation; but they are not homologous, for they do not occupy the same position with reference to the other structures of the hemisphere. The cortex hippocampi and fascia dentata have in Echidna grown downward through the primordium hippocampi into contact with the precommissural body on the median surface of the hemisphere. The result is that, whilst in the adult reptile differentiated cortex is found in a position which corresponds with the dorsal lip only of the foetal fissura arcuata, in the mammal such cortex

is found on both sides of this fissure, the ventral limb having been differentiated at the expense of the reptilian primordium hippocampi.

DISCUSSION

DIENCEPHALON AND TELENCEPHALON

Since the cerebral hemispheres are formed by a lateral evagination of the walls of the rostral end of the neural tube, a clear understanding of the morphological features of the unevaginated portion of the tube is essential to a correct interpretation of the relations of the parts of the hemispheres.

In the lower (epichordal) parts of the embryonic brain tube, the massive lateral wall is divided by the sulcus limitans into dorsal and ventral laminae, of which the former (alar or epencephalic plate of His) is devoted primarily to the receptive functions and the latter (basal of hypencephalic plate of His) to the efferent or effector functions. Correlation tissue of the formatio reticularis type is developed in both laminae, afferent elements predominating in the dorsal and efferent elements in the ventral lamina. Similarly, the descending fibers of the great longitudinal conduction paths tend to develop in the ventral lamina and the ascending fibers in the dorsal lamina; but in the upper (prechordal) parts of the neural tube, this relation is disturbed by massive correlation centers.

The absence of peripheral motor nerves rostrally of the midbrain involves the great reduction of the ventral lamina in this region. Accordingly, the sulcus limitans disappears in the diencephalon. In the human embryo (His) it appears to end in the preoptic recess, which would imply that the chiasma ridge and hypothalamus belong tn the ventral lamina. And such indeed has been clearly shown to be the case, though with great secondary distortion due to the absence of the somatic motor nuclei and the invasion of the residual motor coördination tissue by other elements, chiefly of the visceral type (see Johnston, '09, p. 517).

From the absence of the ventral lamina rostral to the chiasma ridge, it follows that the remaining parts of the diencephalon

and telencephalon belong to the dorsal lamina, this lamina curving around from the dorsal to the ventral surface, so as to form the whole of the rostral end of the lateral wall of the neural tube at the time of the closure of the neuropore (cf. the diagram of the structure of a hypothetical vertebrate ancestor, fig. 72).

The optic vesicle is evaginated from the dorsal lamina at a very early age, this process often beginning before the closure of the neural tube. After the closure of the tube, a similar evagination occurs from its rostral end to form the cerebral hemisphere. The optic evagination does not involve the extreme dorsal part of the dorsal lamina, but includes tissue ventral to the epithalamus and rostral to the thalamus (cf. fig. 72).

The withdrawal of this part of the massive lateral wall into the optic vesicle leaves an area at the site of the future di-telencephalic fissure (margo thalamicus, fig. 82, *2–2*) relatively free from proliferating nuclei. The origin of this fissure, which is limited to the dorsal part of the wall, is explained by Johnston ('09, p. 516) as due to the evagination of massive tissue from this region into the optic vesicle.

The telencephalon of all existing vertebrates consists of a primitive unpaired vesicle, the telencephalon medium, which is a remnant of the first segment of the primitive neural tube, and of secondarily evaginated hemispheres. As we ascend the phylogenetic series, the hemispheres develop progressively and the median vesicle regressively

Comparative considerations suggest that in the earliest phylogenetic stages the evagination of the cerebral hemispheres included only the primary and secondary olfactory centers. The olfactory bulb is, doubtless, the oldest part of the hemisphere. All of its other elements have entered subsequently by the enlargement of the original evagination to include adjacent parts of the wall of the neural tube and the further differentiation of these parts *in situ*. A part of the primordial secondary olfactory center, however, remains permanently in the telencephalon medium of all vertebrates, viz., the preoptic nucleus; and in higher vertebrates the secondary olfactory fibers for this nucleus are partially or wholly replaced by fibers of the third or higher orders.

In cyclostomes another and smaller part of the secondary olfactory center remains permanently in the dorsal wall of the telencephalon medium. There is here a dorso-median ridge termed by Johnston ('02) the epistriatum and by other authors the dorsal part of the praethalamus, the rostral end of which is apparently telencephalic. That is, the evagination of the cerebral hemisphere at first, like that of the optic vesicle, involves neither the extreme ventral nor the extreme dorsal part of the lateral wall of its segment. Fig. 72 is a picture of the probable relations in a hypothetical vertebrate ancestor, in which neither the optic vesicles nor the cerebral hemispheres have evaginated from the neural tube. The tissue which gives rise to the optic vesicle and retina in true vertebrates is indicated at *R*. Its dorsal position is based on Johnston's discussion ('09, p. 479). At *O* is shown the tissue which evaginates in vertebrates to form the olfactory bulb. It is surrounded by secondary olfactory tissue more or less of which is evaginated into the cerebral hemisphere in different vertebrates. The dorso-median di-telencephalic ridge (*d.m.r.*) is a part of this secondary olfactory center, as is also the ventro-median olfactory nucleus (*n.olf.v.m.*). All parts of the secondary olfactory nucleus are connected by fiber tracts with both the hypothalamus and the epithalamus.

The recurving of the dorsal lamina around the rostral end of the ventral lamina brings a part of the secondary olfactory area into immediate contact with the hypothalamus, which as pointed out above (p. 466), is occupied chiefly by visceral motor correlation tissue. The hypothalamus, accordingly, becomes the great avenue of discharge for olfactory reflexes of the visceral (interoceptive) systems and the center for their coördination, a relation which is preserved with great constancy throughout the whole series of vertebrates.

The epithalamic connection of the secondary olfactory area has a quite different significance. I have elsewhere ('08) commented on the fact that the sense of smell, unlike the chemical senses in general, functions both as an interoceptor and as a distance receptor. It is the latter function which requires intimate relations with the exteroceptive centers of the dorsal part of the thal-

amus. What the exteroceptive functions of the epithalamus were in primitive vertebrates it is now difficult to determine, for there have doubtless been important changes of function here correlated with the degeneration of the epiphysis and parietal organ. But we know that in some existing lower vertebrates there is a broad fibrous connection between all parts of the epithalamus on the one hand and both the optic centers and the underlying central grey of the thalamus on the other hand, so that the whole epithalamus is now very probably a correlation center for olfacto-optic and olfacto-somaesthetic impulses, mainly no doubt related with the reflexes of feeding and oral sensibility, as Edinger has suggested. The efferent path from this exteroceptive olfactory center passes to the ventral motor lamina by the shortest possible path through the fasciculus retroflexus of Meynert.

The remainder of the thalamic part of the dorsal lamina is devoted to non-olfactory somatic sensory correlations, and immediately contiguous with these centers motor correlation tissue of the ventral lamina type is differentiated forward beyond the primary limits of the ventral lamina, *i.e.*, beyond the rostral end of the sulcus limitans, thus ultimately obliterating this sulcus in the rostral part of the diencephalon. The sulcus diencephalicus medius marks the dorsal boundary of this forward extension of the somatic motor correlation tissue and therefore is a secondary extension of the sulcus limitans, though not a part of it. Thus are formed the rostral end of the pars ventralis thalami and, farther forward, the corpus striatum complex.

That this division of the thalamus by the sulcus limitans and sulcus medius into dorsal afferent and ventral efferent parts is fundamental and characteristic of the vertebrate phylum as a whole is shown by a survey of the comparative anatomy and embryology of the thalamus, though it is often secondarily obscured in the adult. It is very evident in the generalized fishes, and, as I have shown in this paper, in both embryonic and adult amphibians and reptiles. Ramón y Cajal has clearly demonstrated the same relation in the rodents ('04, p. 774). It is equally clear in early human embryos, as will appear beyond (p. 475). It has, in fact, long been recognized that the rostral end of the

sulcus limitans (sulcus Monroi of Reichert) is a landmark of prime importance embryologically and Mihalkovics ('77, p. 70) in his discussion of the relation of the diencephalon to the hemispheres seems to include both my sulcus medius extending back from the interventricular foramen and the sulcus limitans extending back from the preoptic recess in his sulcus Monroi. His applies the latter term to the rostral end of the sulcus limitans only. Mihalkovics clearly describes the forward extension of the ventral lamina into the lateral wall of the hemisphere; but neither he nor his followers seem to have fully appreciated the significance of the direct passage of the great olfactory radiation into the hypothalamus and the morphological significance of the latter as a motor correlation center derived from the ventral lamina of the neural tube.

In the cyclostome brain the optic vesicles have been completely evaginated and the evagination of the cerebral hemisphere has advanced scarcely beyond the first step, viz., the outgrowth of primary and secondary olfactory centers.

Through the kindness of Dr. Charles Brookover I have been able to study a carefully prepared series of transverse sections through the head of a 120 mm. specimen of Ichthyomyzon concolor (Kirtland) stained with haematoxylin, and I present herewith (figs. 73 to 81) a series of sketches from these preparations to illustrate the relations of the diencephalon and telencephalon. This material by itself is of course insufficient for such an analysis, but the descriptions of Sterzi, Johnston and others enable us to supplement these observations and to present the following interpretation.

The sulcus limitans is not clearly preserved in the diencephalon of this specimen. In the caudal part of the diencephalon (fig. 81) the dorsal (subhabenular,) medial and ventral diencephalic sulci are seen substantially in the same relations as in the Amphibia. The ventral sulcus is interrupted by the chiasma ridge (figs. 80 and 73), but the medial sulcus continues rostrad as far as the interventricular foramen (figs. 79, 78, 77). The relations of the diencephalon and telencephalon as seen in a reconstruction of the median section are shown in fig. 73.

A study of Sterzi's description ('07) of the development of allied species of Petromyzon shows that the locus of the velum transversum is at or near the point marked *v* in fig. 73. Adopting Johnston's definition ('09) of the di-telencephalic boundary, fig. 78 lies very near the plane of this boundary, the postoptic recess extending somewhat farther forward and the evaginated hemispheres farther backward.

The hypothalamus is separated from the pars ventralis thalami by the sulcus ventralis and the large chiasma ridge. The nucleus preopticus is very large and it extends forward above the chiasma ridge to the lamina terminalis in relations practically identical with those of the ventro-median olfactory nucleus of the hypothetical primitive type (fig. 72, *n.olf.v.m.*). There is no sulcus separating it from the overlying pars ventralis thalami and the pars ventro-lateralis hemisphaerii; this boundary, however, can be determined by the internal structure (figs. 77, 78). None of this column is evaginated into the hemisphere. The pars ventro-medialis hemisphaerii, therefore, does not exist as such.

The hemispheric evagination is composed of the whole of the primary olfactory nucleus (bulbus olfactorius) and of a part of the secondary nucleus; viz., of parts which correspond rather closely with the regions marked *O* and *sec. olf.* in fig. 72. The primary nucleus forms the rostral part of the hemisphere and the secondary nucleus (area olfactoria of Johnston) the caudal part, the two being separated by a deep sulcus. The evagination of the hemispheres is strictly lateral; they extend a very short distance rostral to the lamina terminalis, but somewhat farther caudad. The backward movement of the secondary olfactory nucleus causes it to overlap the pars ventralis thalami as seen figs. 78 and 79. The motor correlation tissue represented by the latter part has advanced out a very short distance into the telencephalon, and its relations to the interventricular foramen and the other parts are essentially similar to those of the eminentia thalami and corpus striatum of young urodele larvae.

The sulcus medius extends forward to the interventricular foramen. The foramen lies some distance caudad of the lamina terminalis, from which it is separated by the rostral end of the

dorso-median ridge (figs. 73 to 76). The pars ventralis thalami passes over into the pars ventro-lateralis hemisphaerii (primordial corpus striatum) with but little change of structure. The rostral boundary of this efferent correlation tissue cannot be fixed definitely; doubtless in that region there is a gradual transition into the adjacent secondary olfactory nucleus.

The habenula is highly differentiated and separated from the other parts of the diencephalon by a sharp subhabenular sulcus. Extending forward from the habenula, bordering the taenia thalami, is the massive dorso-median ridge which crosses the site of the embryonic velum transversum into the telencephalon to end at the neuroporic recess in front of the foramen (fig. 73). This ridge is better developed in Lampetra wilderi (the epistriatum of Johnston, '02) than in most other petromyzonts, but in all cases is an evident structure. In the diencephalon of Ichthyomyzon there is no clearly marked boundary between the dorso-median ridge and the pars dorsalis thalami, these structures being less clearly separate than in Lampetra. On the basis of internal structure I have indicated somewhat arbitrarily a boundary by the dotted lines *s.d.*, on fig. 73. The telencephalic part of this ridge is similarly imperfectly separated from the evaginated hemisphere (figs. 75, 76, 77).

The cerebral hemisphere of the petromyzonts, accordingly, contains no representative of the amphibian pars ventro-medialis and a very small pars ventro-lateralis. By far the larger part corresponds with the amphibian pars dorso-lateralis and olfactory bulb, which are the direct forward extensions of the pars dorsalis thalami. The rostral end of the dorso-median ridge receives fibers from the olfactory bulb; it is therefore a part of the general secondary olfactory nucleus. It is traversed for its whole length by fibers of the tractus olfacto-habenularis. Tretjakoff ('09) has shown that these fibers give off collaterals into the dorso-median ridge (his prethalamus) and that some of them end in the thalamus under the habenula. This shows that the epithalamus and dorso-median ridge of cyclostomes are as imperfectly differentiated functionally as structurally from the pars dorsalis thalami.

The diminution in the size of the rostral end of the pars dorsalis thalami is due to two causes; first the evagination of the optic vesicle from this region, and second to the fact that almost the whole of the cerebral hemisphere has been evaginated from the extreme rostral end of this column.

In Ammocoetes, we are told by Tretjakoff that fibers from the parapineal organ, after decussation in the superior commissure, end in the prethalamus. He therefore assumes that this structure (the epistriatum of Johnston, our dorso-median ridge) is a correlation center for sensory impressions from the parapineal organ with others from the olfactory organ. It also receives a hypothalamic tract according to Johnston. Its efferent path in Ammocoetes, as in Lampetra, is by way of the corpus striatum. It seems very probable to me that in higher animals, in which the sensory function of the pineal organs is reduced, the caudal (diencephalic) part of this dorso-median ridge suffers corresponding reduction, while the telencephalic end is preserved on account of its olfactory connection, and is ultimately evaginated into the cerebral hemisphere to become the primordium hippocampi.

Johnston teaches ('02) that the dorso-median ridge lies wholly within the telencephalon. Other students of cyclostomes who have discussed the matter agree that it is wholly diencephalic (Schilling, '07; Sterzi, '07; Kappers, '08; Tretjakoff, '09). As appears from the preceding discussion, I think it clearly extends across the di-telencephalic boundary, in this resembling the other parts of the diencephalon of cyclostomes, which pass forward without interruption into the telencephalon.

In a recent paper Johnston ('10, p. 147) claims that the lateral attachment of the velum transversum to the massive side walls of the brain in several types of fishes lies far caudad of its position in the mid-line, near the habenula, so that much of the border which has been considered by most authors as taenia thalami is really in these forms telencephalic and therefore comparable with the taenia fornicis of Amphibia and higher forms. The data so far available do not seem to me to support this interpretation; but from the standpoint of this discussion it is not a matter of great importance. For if the four longitudinal columns of which the

wall of the diencephalon is composed extend in the primitive vertebrates across the di-telencephalic boundary, as I have described them, without fundamental morphological change, then the differences between Johnston and the other authors as to the exact position of this boundary involve no necessary change in the fundamental morphology.

On account of the very small degree of evagination of the cerebral hemisphere in cyclostomes the di-telencephalic fissure is shallow and the pars dorsalis thalami passes over without interruption into the lateral wall (lobus olfactorius) of the hemisphere. Moreover this fissure does not extend upward to the mid-dorsal line and thus the dorso-median ridge is able to pass continuously from one segment to the other. In higher vertebrates this fissure extends dorsally up to the site of the velum transversum and it is so deep as to interrupt the continuity of both the ridge and all other massive tissue of the pars dorsalis thalami with their telencephalic representatives. Intermediate conditions will probably be found in the lower fishes. In amniotic vertebrates the separation is made still more complete by the development of the posterior chorioid fold of the hemisphere.

In the urodele (see fig. 22) most of the telencephalon except the nucleus preopticus is evaginated into the hemisphere. This nucleus is, however, very large. The corpus striatum complex extends backward from the ventro-lateral part of the hemisphere for a short distance into continuity with the pars ventralis thalami and from the same part the eminentia thalami projects forward into the wide interventricular foramen. The diencephalic part of the dorso-median ridge is rudimentary and the telencephalic part has greatly enlarged to form the primordium hippocampi. We shall return to the internal morphology of the amphibian brain on a later page. At this point I wish to direct attention to a few features of the early development of the human brain.

The dorso-median ridge is evident in a form very similar to that of cyclostomes in the human embryo Br 3, of about four weeks, figured and modeled by His. Fig. 82 is drawn from Ziegler's reproduction of the His model with lettering taken from sketches of the same model in the last paper published by Professor His

('04, figs. 34 and 35). The telencephalic part only of this ridge is present, being seen in the model, but not in this figure, on each side of the median plane along the dorsal part of the margo reuniens (fig. 82, *1*, *1*, *1*.). At this stage it is not involved in the evagination of the hemisphere. A side view of this model showing this feature is published by His ('04, p. 58, fig. 36). In older human embryos, as in most gnathostomes, the telencephalic part of this ridge is fully evaginated into the cerebral hemisphere. It is incorporated into the primordium hippocampi, to whose morphological interpretation we shall return.

The diencephalic part of the dorso-median ridge belongs in the epithalamus. Its fate in gnathostomes is obscure. It appears to be represented in the subhabenular tissue (cf. the Ziegler reproductions of the His models, nos. 1, 4, and 7).

In the four weeks human embryo (fig. 82) the unevaginated part designated by His *C.s.* evidently includes both the corpus striatum and the preoptic nucleus, as well as a part of the tissue which when evaginated will form the precommissural body; that is, it includes the ventro-medial parts of the definitive hemisphere. The evaginated part includes the remainder of the rhinencephalon and the pallium. In fig. 82 the dotted line *1–2–2–1* marks the limit of the hemisphere evagination and the line *3* the site of the future sulcus medius, which appears in the embryo of about 4½ weeks, after the narrowing of the interventricular foramen has begun (see His, '04, fig. 38 and the Ziegler model of embryo Ko). The sulcus medius in the 4½ weeks embryo extends backward to the sulcus limitans and separates the dorsal and ventral parts of the thalamus in the amphibian fashion.

The line *2–2* (fig. 82) marks the beginning of the di-telencephalic fissure, which remains membranous throughout life and connects the epithalamus and pars dorsalis thalami with the pallium of the hemisphere.

I have examined in this connection a large number of series of mammalian embryos of different species and adult brains of the opossum, Didelphys, and of the rat and conclude that the morphological generalizations reached in the preceding discussion find ready application in these brains without any fundamental change.

Since the details of this application in no way modify the general conclusions, I shall not take them up in this contribution, save for one further point, in which the very recent literature is in some confusion, growing out of a failure to effect a correct analysis of the most rostral part of the diencephalon.

Johnston in a series of very important papers (see especially '06, p. 308, and '09, p. 522) has described the primordial hippocampus (his dorsal part of the epistriatum in fishes) as directly continuous with a structure in the unevaginated telencephalon medium which he terms the caudal part of the epistriatum. We have seen that the latter structure in amphibians and reptiles borders the taenia thalami behind the velum transversum and is therefore wholly diencephalic, and that two and sometimes three parts of the diencephalon are represented within it. Its most ventral part is the rostral end of the pars ventralis thalami, or eminentia thalami, bounded above by the sulcus medius. Its dorsal part may be formed by the pars dorsalis thalami in mammals, or by the epithalamus (fig. 18) or by both of these structures (fig. 49). We have seen further that the massive connection of the hippocampal formation with these structures as seen in Amphibia and some Reptilia is secondarily acquired and is not of great morphological significance.

The mammalian structure corresponding to Johnston's caudal part of the epistriatum was first clearly described for foetal Ornithorhynchus by Elliot Smith ('96) under the name paraphysis. A study of Elliot Smith's descriptions and figures in comparison with embryos of eutherian mammals makes it evident that this structure is nothing other than the rostral border of the diencephalon adjacent to the taenia thalami, and this has since been expressly stated to be the case by Professor Smith himself ('08, p. 535). Figures 9, 10, 11 and 12 of the original memoir ('96) give relations of the pars ventralis and pars dorsalis thalami, sulcus medius and sulcus ventralis, almost identical with those of embryonic reptiles (cf. my fig. 49). There is, therefore, no direct morphological relationship between the "paraphysis" of Elliot Smith's earlier description and the hippocampal formation.

Now returning to the amphibian brain, let us examine a section

taken transversely through the diencephalon. The morphological transverse section here is inclined to the long axis of the brain as indicated by the line *A–B* of fig. 22. The chief features of such a section through the rostral end of the diencephalon of both Urodela and Anura are indicated in fig. 83, which shows that the walls of the neural tube here are composed of ten longitudinal columns or laminae. Besides the unpaired membranous roof plate and floor plate, there are four ridges on each side, the epithalamus, the pars dorsalis thalami, the pars ventralis thalami and the hypothalamus, separated by the dorsal, median and ventral sulci of the diencephalon.

The mode of development in early embryos shows that the eight massive columns are not produced by a passive plication of the walls due to extrinsic forces; but that each column is a center of more active proliferation of neuroblasts than the intervening sulci, and has, therefore, doubtless been differentiated under the influence of definite functional requirements.

The roof plate and floor plate converge into the lamina terminalis, where of course they end. The four massive columns on each side converge into the interventricular foramen, and in larvae with wide foramina and adult urodeles they may be followed through the foramina into the evaginated hemispheres. Bearing in mind the fact that during development the roof plate and floor plate retain permanently their primitive attachments to the lamina terminalis, and that it is only the massive lateral columns which are evaginated into the hemispheres, it clearly follows that these columns of the diencephalon are continued into the hemispheres in the form shown by the accompanying diagram (fig. 84), the zona limitans lateralis representing the locus of the sulcus medius and the zona limitans medialis the line of union of the dorsal and ventral columns in the lateral evaginations rostral to the fusion of the roof plate and floor plate in the lamina terminalis.

The massive dorsal columns 1 and 2 are interrupted by the di-telencephalic fissure and the telencephalic portions are fully evaginated into the hemispheres. In the cyclostomes column 2 is evaginated to form the area olfactoria of Johnston, while column 1 remains unevaginated in the telencephalon medium as the

so-called prethalamus ("epistriatum" of Johnston) and is therefore not interrupted by the di-telencephalic fissure. Column 3 is partially evaginated in cyclostomas and urodeles and fully so in higher animals. Column 4 is not evaginated at all in cyclostomes. In the amphibians about half of the telencephalic part of this column remains in the telencephalon medium as nucleus preopticus, while the remainder is evaginated to form the pars ventro-medialis of the hemisphere. In the higher forms the evaginated part increases at the expense of the median part until in man the small supraoptic nucleus is all that remains of the latter, while the former includes the precommissural body, septum pellucidum, tuberculum olfactorium, etc. In view of the relations of the olfactory bulb to the other parts of the hemisphere in early phylogenetic stages, as discussed above (p. 468), it is probable that this, the oldest part of the hemisphere, is terminal with reference to each of the other four parts of the secondary or evaginated part of the telencephalon. This, of course, does not imply that the olfactory bulbs represent the anterior end of the primitive neural tube, for the latter lies in the lamina terminalis or below it, probably in the preoptic recess. In higher vertebrates the olfactory bulb seems to belong to the pars ventro-medialis hemisphaerii; but this peculiarity has been acquired secondarily by reason of the preponderating importance of the efferent tract to the hypothalamus, as discussed on p. 468.

The adult frog presents us with a typical picture of the fully evaginated cerebral hemisphere reduced to its lowest morphological terms. A recapitulation of the morphological characteristics of its subdivisions follows:

(1) The olfactory bulb. This we have considered in the immediately preceding paragraphs.

(2) The ventro-medial part. This is primarily a secondary olfactory center, receiving the tractus olfactorius ventro-medialis and is directly continuous behind and genetically related with nucleus preopticus of the telencephalon medium and the hypothalamus. The hypothalamus is an important diencephalic correlation center and in early phylogenetic stages it sends ascending fibers into the pars ventro-medialis of the hemisphere. These

terminate in a special olfacto-hypothalamic correlation center, the nucleus medianus septi, which is the most important component of the precommissural body and which in Amphibia effects associational connections with all other parts of the hemisphere. The descending tractus olfacto-hypothalamicus and the ascending hypothalamo-septal tract constitute the most important components of the medial forebrain tract. The whole pars ventromedialis is also related with the habenula by way of the stria medullaris. The latter is an olfacto-somatic correlation path, while the larger hypothalamic connection is for olfacto-visceral reflexes.

(3) The dorso-lateral part. This also is primarily a secondary olfactory center, receiving the tractus olfactorius dorso-lateralis. It is connected by associational tracts with the dorso-medial part and much more intimately with the ventro-lateral part, from which it is very imperfectly separable in urodeles and frog tadpoles. It is represented in mammals as one of the components of the pyriform lobe.

(4) The ventro-lateral part. Secondary olfactory fibers reach this part, but in smaller number than the dorsal-lateral part. In the frog these come chiefly from the bulbulus accessorius and are distributed to a special grey center, laterally of the lamina terminalis, which has by some recent authors called been corpus striatum. The remainder of this part is characterized by the great lateral forebrain tract, which includes descending projection fibers from both lateral parts to the pars ventralis thalami and lower regions and ascending projection fibers from the pars dorsalis thalami, including the general sensory nuclei and the corpus geniculatum laterale, and from the colliculus inferior to both lateral parts. It is the great somatic or exteroceptive projection tract for the hemisphere and passes back directly into the pedunculus cerebri system; i.e., it is the precursor of the striatal and internal capsule fibers of mammals. The ventro-lateral part includes within it the materials out of which the mammalian corpus striatum is developed, though the striatum as such is not yet differentiated.

The two lateral parts of the hemisphere, then, are the direct

continuation of the corresponding parts of the neural tube in the diencephalon. The ventral part clearly continues the pars ventralis thalami, viz., the efferent center lying below the sulcus limitans. The dorsal part is the telencephalic representative of the pars dorsalis thalami, or somatic sensory center above the sulcus limitans.

(5) The dorso-medial part, or primordium hippocampi. This receives in all vertebrates olfactory fibers of the second or third order at its rostral end and it is connected by association fibers with the ventro-median and dorso-lateral parts. It gives rise to the fibers of the commissura hippocampi, which in all Amphibia is separated into a commissura pallii anterior and a commissura pallii posterior. The columna fornicis passes from it to the hypothalamus by the usual course, from the rostral end of the primordium dorsal and rostral to the interventricular foramen and anterior commissure, thence through the lamina terminalis.

In reptiles (and possibly to a very slight extent in Anura) cortex hippocampi is differentiated within this part. In mammals it becomes the hippocampus. In cyclostomes, and perhaps in some other fishes, the dorso-median part of the hemisphere is connected by a massive bridge of grey matter with tissue in the epithalamus closely associated with the habenula. In Amphibia and Amniota, this connection is lost. Nevertheless, it seems probable that the primordium hippocampi is topographically the telencephalic extension of the epithalamus. The establishment of different functional connections of these structures has led to a wide divergence of form and significance.

The course of the commissural fibers of the primordium hippocampi shows remarkable variations. In mammals these fibers pass forward above the interventricular foramina to cross in or above the lamina terminalis. In ganoids and teleosts, they go below and behind the foramina. In Selachians, Amphibia and some reptiles they separate into the commissura pallii anterior and posterior. In the amphibians, the anterior part passes forward behind and below the foramina to cross in the lamina terminalis and the posterior part backward by way of the stria medullaris to cross in the superior commissure. In reptiles the anterior

part passes forward above the foramina to cross in the lamina terminalis and the posterior part crosses directly through the velum transversum. In selachians the anterior part passes forward above the foramina to cross above the lamina terminalis and the posterior part passes backward to cross in the superior commissure, as in Amphibia (Johnston, '10).

The peculiar aberrant courses shown by a comparative study of the commissura hippocampi (and many similar illustrations might be given) suggest that these fiber systems are in the phylogeny functionally determined and that the particular pathways selected by the fibers in passing from origin to termination are variable depending in part on the mechanics of ontogenetic and phylogenetic development.

In this connection it should be borne in mind that the commissural fibers of the primordium hippocampi develop late in the ontogeny after the chief structural differentiation of the brain wall has far advanced. In macrosmatic species, like the selachians, where the secondary olfactory nuclei are greatly enlarged at the rostral end of the hemisphere (particularly the ventro-median nucleus) the primordium hippocampi attains its greatest development at its rostral end and some commissural fibers take the shortest path across the area of fusion of the primordia in the lamina supraneuroporica. In reptiles the same factors operate to some extent and in addition the rapid backward growth of the posterior poles of the hemispheres and the consequent early development of the posterior chorioidal fold tend to impede the subsequent growth of fibers from the hippocampus downward into the lamina terminalis behind the foramina. Accordingly they grow forward above the foramina into the lamina terminalis. But in some cases (lizards) where the anatomical configuration is favorable some of these fibers from the posterior pole find a shorter path and cross in the velum transversum to form the commissura pallii posterior. In ganoids and teleosts, on the other hand, the centers containing the primordium hippocampi lie far back in the telencephalon medium and their commissural fibers grow forward below the ventricle into the lamina terminalis by the shortest path. Similarly in the urodeles, especially the early larvae, the

primordium hippocampi lies chiefly in the posterior pole and the long septum ependymale cuts off the dorsal path to the lamina terminalis; accordingly the commissural fibers take the same path as in teleosts. But this by no means implies a genetic relationship between teleosts and Amphibia or that the Amphibia are more closely related to the teleosts than they are to the reptiles. From the standpoint of evolution our interest centers not in the variations of these commissural fibers, which arise late in the ontogeny and have small phylogenetic significance, but rather in the changes in position of the grey masses from which they spring, which in this case concern merely their relative cephalo-caudal position—a factor easily varied in the course of phylogeny by changes in the composition of the sensori-motor reflex pattern or action system of the species.

In reptiles and mammals the five parts of the cerebral hemisphere as above summarized have very different histories. The olfactory bulbs suffer no fundamental change, nor does the pars ventromedialis save for the differentiation within it of special nuclei, chiefly under the influence of the descending columns of the fornix and of the corpus striatum. They entro-lateral and dorso-lateral parts give rise to the corpus striatum and pyriform lobe, which remain in very intimate relation from the beginning to the end of the phylogenetic history.

The nervus terminalis has not been considered in this paper for the reason that we still lack sufficiently precise data to effect its morphological interpretation. Previous descriptions indicate that it is related probably with the precommissural body. Mr. P. S. McKibben in this laboratory has in preparation a report on the central relations of this nerve in urodeles, in which he finds it related with the whole of the pars ventro-medialis hemisphaerii, still more extensively with the nucleus preopticus and also with the hypothalamus. But we have as yet no sucffient data for a determination of its functional connections and therefore its morphology remains obscure.

The relations of the amphibian dorso-medial and dorso-lateral parts to the cerebral cortex will be considered on a later page; but first we must recur to the relations of the precommissural body.

THE PARATERMINAL BODY

Elliot Smith has applied the term precommissural body, or paraterminal body, to certain structures associated with the lamina terminalis and septum in the median wall of the hemisphere; and in his penetrating analysis of the relation of the hippocampus to the remainder of the cerebral hemisphere ('03, p. 489) he emphasizes the intimate relation of the hippocampus to the paraterminal body and adds, "a study of the relations of the *primordium hippocampi* to the paraterminal body in the Ichthyopsida lends support to the view that the hippocampus may be merely the specialized upper part of the primitive paraterminal body."

In testing the validity of this hypothesis, it at once appeared that the diponan and reptilian paraterminal body, as defined by Elliot Smith, is a two-fold structure, whose components are probably distinct in origin and subsequent evolution.

(1) The ventral component of the paraterminal body lies within the pars ventro-medialis of the hemisphere and is a basal olfactory center, developed originally as a terminal nucleus of the ventral division of the tractus olfactorius medialis, to which have been added olfactory fibers of the third order, ascending fibers from the hypothalamus and other connections. Within it is differentiated the nucleus medianus septi, some of whose cells form the "bed" of the commissures of the lamina terminalis. Others of these cells grow upward along the course of the fimbria to form the pars fimbrialis septi (Kappers), while still others are related to the fibers of the commissura pallii posterior in certain reptiles. This component is always morphologically ventro-medial. I recommend that the use of the term precommissural body be limited to this component.

(2) The dorsal component of the paraterminal body, as described by Elliot Smith for Lepidosiren, belongs in the dorso-median wall of the hemisphere and in some urodeles (particularly larvae) the corresponding structure is almost completely separated from the ventral component by the membranous septum ependymale. It is, in fact, the primordium hippocampi. The hippo-

campus arises exclusively at the expense of this structure, and from the Amphibia onward the gradual transformation of the primordium hippocampi into cortex hippocampi can be easily followed until in the mammals it is practically all so consumed. In reptiles the dorso-medial cortex arises from this primordium; but in old embryos there is always an unspecialized remnant of it left ventrally. This sometimes is preserved in the adult; in other cases it is filled with fimbria fibers and loses its identity. When present, it is generally separated from the overlying cortex hippocampi by a slight superficial sulcus, which is the first rudiment of the fissura hippocampi (fissura arcuata of mammalian embryology). Attention is especially called to the fact that this fissure develops *within* the hippocampal formation and should not be confused, as has often been done, with the phylogentically older fissura limitans hippocampi, which separates the hippocampal formation from the underlying precommissural body (see p. 464).

There is no evidence in any vertebrate that any cells of the pars dorso-medialis have been derived from the pars ventro-medialis. On the contrary, these two parts appear to have differentiated from primordia which have retained a structural independence which goes back to the primary unevaginated neural tube. In this I support the opinion of Adolf Meyer ('95) and of Ramón y Cajal ('04, p. 1051). The evidence for this conclusion has been fully presented above. Here we point out merely: (1) that the lower Amphibia, particularly in the early larval stages, show a wide separation of the dorsal and ventral parts of the median wall by a membranous septum such as to forbid the migration of cellular elements from the one to the other, except at their rostral ends where they converge merely to receive their olfactory tracts; (2) that the primordium hippocampi attains its characteristic amphibian structure while this septum is still membranous; (3) that the massive septum of Anura develops wholly at the expense of the underlying nucleus medianus septi; (4) that even here, where at no stage a membranous septum ependymale is present, the septum and primordium hippocampi are separated at all stages subsequent to the differentiation of their

grey matter by a clearly marked zona limitans relatively free from nuclei. Each of these parts of the cerebral wall is differentiated at the expense of its own ventricular grey and neither is in the ontogeny to any extent derived from the other so far as its cellular material is concerned. It is my opinion that the same is true when we consider the origin of the primordium hippocampi phylogenetically.

It is, on the other hand, equally evident that the physiological motive which has led to the further differentiation of tissue within the rostral part of the primordium hippocampi in Anura is the functional connection with the underlying precommissural body. The size and structural complexity of the primordium hippocampi and of the precommissural body vary in relation to each other and the richness of the connecting fiber systems. The precommissural body serves as a way-station between the primary olfactory centers and the hypothalamus and hippocampus, as the avenue of discharge into the hippocampus of ascending fibers from the hypothalamus and as a pathway for efferent impulses from the hippocampus having a collateral connection from the fornix with the precommissural cells and discharging chiefly into the hypothalamus. In the Amphibia these functions are imperfectly localized, but in mammals the researches of Cajal show that the nucleus medianus septi is devoted chiefly to the olfacto-hypothalamic connections and the nucleus lateralis to the collateral fornix fibers.

Ramón y Cajal ('04, p. 1050) is of the opinion that the mammalian corpus striatum is a center for the reinforcement (by means of collateral discharge) of the impulses sent out from the cortex by the efferent projection fibers of the internal capsule and that the nuclei of the septum (particularly the lateral nucleus) bear a similar relation to the fibers of the commissura hippocampi and fornix. He figures in the rat with great clearness collaterals both from the commissure and from the fornix to the cells of the septum, especially its lateral nucleus. Elliot Smith ('10) has arrived at a similar conclusion. Though clearly this collateral relation with the efferent fibers from the hippocampal formation was not the primary functional motive in the differentiation of the septal

nuclei, it is probably the explanation of the great development of the lateral nucleus in reptiles and lower mammals. The close association of cells of the nucleus medianus septi with both the anterior and posterior pallial commissures of reptiles, and the migration of other elements upward into the fimbria as pars fimbrialis septi of amphibians are doubtless similarly explained, this being a case of neurobiotaxis (Kappers) or the movement of cell bodies toward the source from which their stimulus is received.

Two other structures in this region of reptiles and lower mammals require a word of comment. The corpus striatum extends around the ventral border of the lateral ventricle into the median wall to form the nucleus accumbens septi (see fig. 43 and Kappers, '08). This is differentiated parallel with the tuberculum olfactorium farther ventrally. These two centers are both concerned with the correlation of the medial and lateral elements of the hemisphere. The tuberculum olfactorium puts the olfactory bulb, septum and hippocampal cortex on the one hand into relation with the striatum on the other hand, the olfactory function predominating; the nucleus accumbens similarly puts the septum into relation with the striatum with the striatal function dominant.

THE MORPHOLOGY OF THE CEREBRAL CORTEX

The simple natural subdivision of the cerebral hemisphere of the frog summarized above (p. 478) is so evident anatomically as to have been commented upon by all students of the subject. In my opinion it gives the key to the functional interpretation, not only of this brain, but of all higher brains. The four chief parts of the hemisphere converge in front into the olfactory bulb. The two ventral parts (pars subpallialis, Gaupp) connect directly with the hypothalamus and pars ventralis thalami respectively. The two dorsal parts (pars pallialis, Gaupp), though morphologically related, as we have seen, to the pars dorsalis thalami and epithalamus respectively, are cut off in forms above the fishes from direct massive connection with the diencephalon by the di-telencephalic fissure. Thus the dorsal parts, which originally belonged

to the secondary olfactory nucleus, are partially isolated physiologically by the interruption of the ancient path of longitudinal correlation. Ascending fibers have grown forward in the mean time from the hypothalamus into the ventro-median part of the hemisphere and from the thalamus into the ventro-lateral part, and these parts became important correlation stations between the olfactory bulb and the hypothalamus and thalamus respectively.

The overlying dorsal parts are profoundly modified by the change in the character of these ventral parts. They receive secondary olfactory fibers from the olfactory bulb in front, but are not directly connected with the great pathways of efferent discharge. They are therefore unfavorably located to serve as organs of the direct and stereotyped olfactory reflexes, but by that very fact are in a favorable position to serve those reactions which involve more extensive correlation and more deliberate response.

The function of the amphibian dorso-lateral part, as of the pyriform lobe of mammals, is evidently the correlation of olfactory with other exteroceptive impressions belonging to the somatic sensory systems. In this complex the olfactory element clearly predominates in the frog and the type of organization is really no higher than that of the ventro-median part in which the olfacto-hypothalamic (and presumably visceral) reflex systems predominate.

The dorso-median part receives a smaller number of direct olfactory fibers. Its other afferent elements come from two sources, partly from the nucleus medianus septi and partly as association fibers from the dorso-lateral part. The rostral end develops under the influence of the olfactory and septal factors chiefly, the caudal end under the lateral influence (olfacto-somatic), and the commissura pallii anterior effects a thorough coördination of these elements, its fibers arising throughout this part and spreading through the whole of the opposite dorso-median and dorso-lateral parts. Efferent fibers leave the medial edge of the dorso-median part for the hypothalamus, and its posterior end for the epithalamus. They leave its lateral edge for the thalamus by way of the lateral forebrain tract.

These relations characterize the dorso-medial part as primordium hippocampi and the cortex hippocampi of higher forms differentiates within it. But in the frog it is clearly much more than an olfacto-hypothalamic correlation tissue. Though structurally very distinct from the dorso-lateral part, the functional connection between these parts is most intimate. If we adopt the interpretation of Elliot Smith ('08, p. 529) that the pars dorso-medialis of the frog brain is the primordium of the mammalian hippocampus and the pars dorso-lateralis that of pyriform lobe, as I think we must, it can only be with the reservation that the homology is incomplete and the parts here are very imperfectly differentiated from each other. All parts of the amphibian hemisphere are under the physiological influence of the olfactory bulb to some extent; *i.e.*, there is no somatic pallium devoted wholly to non-olfactory correlations. On the other hand, there is, I believe, no part of the dorsal or pallial wall of the hemisphere which is not influenced to some extent by the somatic sensory ascending elements in the lateral forebrain tract. The homologies suggested above when given their proper limitations may be expressed as follows: The predominating structural and physiological features of the medial border of the dorsal wall of the amphibian hemisphere are hippocampal in type, while those of the lateral border are those of the pyriform lobe. The dorsal tissue between these borders is undifferentiated. From it the somatic pallium of higher vertebrates arises. It would be an error to consider that the amphibian primordium hippocampi is exactly comparable with the mammalian cortex hippocampi, though more simply organized. On the contrary, it is adapted to serve all the forms of cortical association which the animal possesses—olfacto-gustatory, olfacto-tactile, olfacto-optic, etc.,—but always predominately olfactory. With the differentiation of neo-pallial (*i.e.*, non-olfactory) cortical association centers in mammals, the cortex hippocampi (particularly the fascia dentata) becomes more nearly purely olfacto-receptive and the correlation tissue is separately developed in neighboring association centers of the gyrus cinguli, etc.

The precise history of this differentiation will doubtless be

possible when the internal structure of the reptilian brain is more fully known. It is probable (see above, p. 464) that here the ventral margin of the dorso-median cortex is morphologically and physiologically very similar to the mammalian hippocampus; and that the cortex lateralis, which differentiates over the striatum-epistriatum complex and within the sphere of influence of the lateral olfactory tract, stria medullaris and lateral forebrain tract (tr. strio-thalamicus), is comparable with the pyriform lobe with neopallial (somatic sensory) factors predominating. But the fact that in Lacerta fibers arising from all parts of this cortex pass by way of the fimbria into the commissura hippocampi and fornix (Ramón y Cajal, '04, fig. 852, p. 1103) suggests that, though the reptilian differentiation is far in advance of the amphibian, nevertheless the localization of cortical function is still very imperfect in reptiles and the terms hippocampal cortex and neopallial (somatic) cortex, if used at all, must be employed with the same reservations made above in our discussion of the amphibian pallium, *i.e.*, as designations of spheres of predominant olfactory and non-olfactory sensory-motor coördination within a common matrix.

We conclude, then, that the distinction between neopallium and archipallium (hippocampal formation), while valid physiologically and histologically in higher brains, does not rest upon a difference in the time of their first appearance, for the primordium of the archipallium is not older than that of the neopallium. The earliest primordia of the cerebral cortex performed both functions. Nevertheless, since the olfactory function clearly predominated in this complex in its early phylogeny and since (in correlation with the last point)in Ichthyopsida this primordium occupies the morphological position of the mammalian hippocampus, it is permissible to speak of the common cortical Anlage as primordium hippocampi. Moreover, there is no question that the hippocampal formation reached its full functional maturity earlier in the phylogeny (viz., in the lowest mammals) than did the neopallium, which is apparently now in process of further evolution in the human race. On this ground the term neopallium is justified, even though its simple primordia may be as old as those of the hippocampus.

In this last point I presume Elliot Smith would concur, for in discussing the rudimentary cerebral cortex which occurs in dipnoans he remarks ('08, p. 529), "How much of the pallial formation in Lepidosiren is hippocampus and how much is pyriform lobe or whether, as in the Mammalia, there is any representative of the neopallium interposed between them are all questions which it is impossible to answer. We ought rather to look upon the pallium of Lepidosiren as an area, which is yet unspecialized, the rudiment of that more extensive cortical field which in the Mammalia becomes differentiated into distinct formations." Cf. also his discussion, 1910. The non-olfactory associations of the hippocampus are clearly of great importance even in the mammals, as is proved by the fact that in anosmatic animals, such as the Cetacea, the hippocampus is still extensively and typically developed save for the absence or extreme reduction of its fascia dentata (Hill, '93; Zuckerkandl, '87).

We have as yet no satisfactory definition of cerebral cortex and possibly the attempt to formulate such a definition at this time is premature.

Several recent writers (Johnston, Kappers, Edinger) have attempted to define the cortex hippocampi exclusively in terms of its relation to the olfactory system, viz., as a tertiary olfactory center. But this is a quite insufficient criterion. Johnston in his last contribution to the subject ('09) has emphasized some of these objections and shown that the cerebral cortex, like the other suprasegmental mechanisms, is essentially an organ of *correlation*. Now, the path of conduction for afferent impulses of a single type may have as many synapses interpolated and as many avenues of collateral discharge as you please, without thereby necessarily itself acquiring any significance as a center of correlation. The fundamental architectural motive underlying the evolution of the cerebral cortex is the demand for a center into which two or more *different* afferent types may meet and discharge into a single common final path. Of couse, this is not an exclusive property of the cerebral cortex. What I mean to say is that it is this type of subcortical correlation center which has been elaborated to form the cortex.

In the light of the demonstration in this paper that the division of the cerebral hemisphere of Amphibia (and of higher animals) into dorsal or pallial and basal or subpallial parts is primary and has its morphological basis in the configuration of the primitive neural tube antecedent to the evagination of the hemispheres, I think a provisional formulation of a morphological definition of cerebral cortex is possible. The term was originally applied to the dorsal or pallial superficial grey, as distinguished from ventricular or central grey and from ventral or basal grey of the cerebral hemisphere, and it is still commonly used in this sense. Its application to the ventricular grey (*e.g.*, of cyclostomes by B. Haller, '08) leads to confusion and is objectionable, as is also the designation of superficial grey over the tuberculum olfactorium and other ventral masses as cortex. The classical and prevailing usage finds its justification in the fact that the ventral (subpallial) part of the hemisphere is dominated by efferent pathways for relatively simple direct reflexes (laterally the corpus striatum and lateral forebrain tract, medially the precommissural body and medial forebrain tract) which either directly or by way of the hypothalamus enter into the ventral motor lamina of the neural tube, while the dorsal or pallial part of the hemisphere is the direct continuation of the dorsal lamina of the neural tube and, being only indirectly related with the great efferent centers, is favorably situated to serve the higher non-stereotyped reflexes.

Accordingly, I submit the following definitions:

Pallium telencephali—The dorsal wall of the telencephalon, whether membranous or massive, whether evaginated into the cerebral hemispheres or remaining in the telencephalon medium, being bounded behind by the velum transversum, in front by the olfactory bulbs, laterally by the fissura endo-rhinalis and medially in the evaginated hemisphere, by the fissura limitans hippocampi.

Cortex cerebri—Correlation tissue developed as superficial grey matter within the dorsal (pallial) walls of the cerebral hemispheres.

THE SUBDIVISION OF THE PROSENCEPHALON

Johnston suggests ('09, p. 533) a revision of the BNA subdivision of the cerebrum. Adopting the point of view of his revision, I make the following comments upon his proposals. In discussing the general morphology of the telencephalon, he says ('09, p 519) "The term *hemisphere* is applied in the BNA to each half of the telencephalon. It would therefore include the right or left half of all that lies in front of a plane passing behind the interventricular foramina and the chiasma-ridge."

It is true that in the BNA tables the term hemisphere is applied to each half of the telencephalon *as that term is there defined;* but by treating the hemisphere as the lateral half of the telencephalon *as differently defined* in this paper it seems to me that Professor Johnston has introduced unnecessary and unfortunate confusion.

In an earlier paper ('08a), I adopted and defended the boundary between the diencephalon and the telencephalon as defined in the BNA tables; but Johnston in the paper cited has made it plain that a full knowledge of the comparative embryology of these parts requires a revision of the BNA tables in this respect to accord more nearly with the original usage of His. That is, the telencephalon is not to be regarded as a secondary derivative of the primary segmental neural tube, but as the terminal segment of the tube with its secondary derivatives. Accordingly, the lamina terminalis, preoptic recess and adjacent parts, instead of being assigned to the diencephalon, are regarded as belonging to the telencephalon.

But in the interest of conformity to past usage, as well as anatomical fitness, the term *hemisphere* should be limited to the secondarily evaginated derivative of the primary neural tube and sharply distinguished from the primary terminal segment of the neural tube from which the evaginated portion is derived. That is, the hemisphere should be defined exactly as in the BNA tables; and when we include more in the telencephalon than the hemispheres the added tissue should be given an appropriate name of its own without disturbing the existing definition of the hemispheres.

This is the usage adopted by Sterzi and clearly illustrated in his descriptions of the brains of cyclostomes and selachians (see especially '09, pp. 694, ff.), and also by Tandler and Cantor ('07) in their description of the development of the gecko brain. The unevaginated portion of the first segment of the neural tube and its adult derivatives (ventriculus impar telencephali, aula, recessus preopticus, lamina terminalis, etc.), then, should be called the telencephalon medium, while the hemisphaeria cerebri are the evaginated portions of the telencephalon, as in the classical usage.

This is sound morphology from the standpoints of both embryology and comparative anatomy. The relation of the hemisphere to the telencephalon medium in its earlier stages (both embryonic and phylogenetic) is somewhat similar to that of the retina to the primordial neural tube in the next following segment. In both cases a peripheral sense organ (nose, eye) has called forth in the neural tube massive primary terminal nuclei, whose further enlargement was possible only by an evagination of the wall of the primary tube. An analogous evagination is found in the vagal lobes of cyprinoid fishes, where the hypertrophied gustatory apparatus has called forth a similar response in the medulla oblongata. In the case of the eye the peripheral receptive cells are included in the evaginated tissue (rods and cones of the retina); in the nose the primary receptive cells are independently differentiated peripherally in the olfactory placode. In both cases the centers containing the neurones of the second order are completely evaginated and are located in the retina and olfactory bulb respectively.

In the rhinencephalon the secondary and tertiary olfactory grey are directly continuous with that of the unevaginated primordium and this with diencephalic grey centers farther back. The evaginated portion of the olfactory correlation tissue receives successively more afferent tracts from non-olfactory centers farher back and by the associations here set up the cerebral cortex was gradually elaborated.

It thus appears that, while in cyclostomes and some other lowly vertebrates the hemispheres and primordial telencephalon are related to each other in much the same way as are the primary

and secondary terminal nuclei of the sensory nerves of the medulla oblongata, in higher vertebrates progressively more complex correlation tissue is added to the olfactory association centers and the hemispheres assume their definitive form as the centers for the highest forms of neural function.

In lower vertebrates, particularly some elasmobranchs, the walls of the telencephalon medium are greatly elongated and contain correlation tissue which is incorporated into the hemispheres of higher forms. The nucleus preopticus is the remnant of this unevaginated tissue which is most constantly preserved. This nucleus is continuous rostrally in lower vertebrates with the basal grey of the rhinencephalon of the hemispheres. In teleosts and some ganoid fishes the telencephalon is atypically developed and the hemispheres are very imperfectly differentiated. The lamina terminalis, tela chorioidea and ventriculus impar of the telencephalon are thrust far forward so that the actual evagination includes little but the olfactory bulbs, while the massive correlation tissue which corresponds morphologically and functionally with the greater part of the walls of the hemispheres of some selachians, dipnoans and amphibians lies in the floor of the telencephalon medium.

In view of the data presented in the body of this memoir I would modify Johnston's suggestions as follows:

Mesencephalon
- Pars ventralis—pedunculus cerebri
- Pars dorsalis—corpora quadrigemina

Diencephalon
- Epithalamus
- Hypothalamus
- Thalamus
  - Pars dorsalis
  - Pars ventralis

Telencephalon
- Telencephalon medium
  - Ventriculus tertius pars telencephalica (or ventriculus impar telencephali)
  - Lamina terminalis

Tela chorioidea telencephali
Paraphysis
Chiasma opticum
Commissura postoptica (com. superior Meynerti and com. inferior Guddeni BNA)
Commissura anterior
Nucleus preopticus
Hemisphaerium
Ventriculus lateralis
Pars ventralis
Pars ventro-medialis (characterized by the tractus ventro-medialis hemisphaerii—"olfactory radiations," etc.)
Corpus precommissurale
Tuberculum olfactorium
Pars ventro-lateralis (characterized by the tractus ventro-lateralis—"internal capsule" system)
Corpus striatum
Pars dorsalis s. pallialis
Plexus lateralis
Hippocampus (cortex medialis)
Commissura hippocampi
Lobus pyriformis (cortex lateralis)
Neopallium (cortex intermedius)
Corpus callosum
Bulbus olfactorius

In classifying the lobus pyriformis (gyrus hippocampi and uncus, BNA) as a part of the pallium in the above table there is a certain inconsistency; but the irregularity is inherent in the anatomical facts and will have to be recognized in some other way, if not in this way. Phylogenetically both the hippocampus and the lobus pyriformis take their origin from the primitive area olfactoria. The hippocampus has differentiated far from this original type and such olfactory fibers as enter it are for the most part interrupted by a synapse in the corpus precommissurale. The lobus pyriformis, on the other hand, remains much more

primitive and in all vertebrates receives olfactory fibers directly by way of the lateral olfactory tract. Nevertheless I have shown that from the first appearance of the hemisphere the primordium from which the pyriform lobe developes is clearly separate from that of the sub-pallial parts, and it is generally recognized as showing in mammals some cortical differentiation.

It is difficult to frame any general scheme of anatomical subdivision of the telencephalon which will be readily applicable to all aberrant types, *e.g.*, to the teleosts, where the concentration of all massive tissues in the floor of the ventricle and its subsequent eversion has confused the arrangement of the primary laminae. The above table, like that of the BNA upon which it is based, is drawn up with particular reference to the mammals, whose telencephalon is largely evaginated into the hemispheres. A subdivision based on phylogeny may be suggested as follows:

Telencephalon
- Ventriculus impar telencephali
- Ventriculus lateralis
- Pars ventralis
  - Chiasma opticum
  - Commissura postoptica
  - Commissura anterior
  - Lamina terminalis
  - Pars ventro-medialis
    - Nucleus preopticus
    - Corpus precommissurale
    - Tuberculum olfactorium
  - Pars ventro-lateralis
    - Corpus striatum
- Pars dorsalis s. pallialis
  - Tela chorioidea telencephali
  - Paraphysis
  - Plexus lateralis
  - Pars dorso-medialis
    - Cortex medialis
      - Cortex hippocampi
      - Fascia dentata

Fimbria
Commissura hippocampi
Pars dorso-lateralis (lobus pyriformis)
Cortex lateralis
Cortex intermedius (neopallium)
Corpus callosum
Nucleus olfactorius anterior
Bulbus olfactorius

The relative proportions of these parts which are evaginated to form the cerebral hemispheres will vary as we ascend the phyletic series.

The rhinencephalon as commonly defined crosses the natural boundries set in both of the preceding tables and therefore it cannot be included within them. Nevertheless it, like the term ophthalmencephalon which I recently proposed, stands for a concept which has a certain morphological and physiological value and should be preserved in our nomenclature. I would subdivide it as follows:

1. Bulbus olfactorius, containing the termini of the fila olfactoria, the glomeruli, mitral cells and granules.
2. Nucleus olfactorius anterior, undifferentiated olfactory tissue of the second order, usually closely associated with the bulbus, the two often being both represented in the terminal swelling commonly called the bulbus, and extending backward a longer or shorter distance between the true bulbus and the more specialized parts to be next enumerated.
3. Pars medialis rhinencephali. The olfactory centers in the pars ventro-medialis hemisphaerii (corpus precommissuralis, tuberculum olfactorium, nucleus preopticus, etc.,) and the olfactory part of the hypothalamus.
4. Pars lateralis rhinencephali, includes in fishes the tractus and nucleus olfactorius lateralis and gives rise to the mammalian lobus pyriformis.
5. Pars dorsalis rhinencephali. Includes the dorso-medial olfactory tract and its nucleus in fishes and gives rise to the hippocampal formation of mammals.
6. The habenular nuclei and tracts also should logically be included in the rhinencephalon.

SUMMARY

An examination of the brains of adult amphibians in comparison with embryos of these animals and of reptiles and mammals reveals a simple morphological pattern which is common to the diencephalon and the telencephalon and which rests directly upon the funadmental longitudinal divisions of the early neural tube as defined by His. In the diencephalon the six primary laminae of His (roof-plate, floor plate, dorsal plates and ventral plates) become ten by the division of the dorsal and ventral laminae on each side into two parts, so as to give in addition to the unpaired membranous roof plate and floor plate four others on each side, viz., the epithalamus, pars dorsalis thalami, pars ventralis thalami and hypothalamus. These are functionally defined and are structurally evident in vertebrate embryos generally and in all adult Amphibia (see fig. 22 and pp. 466 ff.).

In the telencephalon the roof plate and the floor plate converge in the lamina terminalis and the massive side walls are more or less completely evaginated to form the cerebral hemispheres. In embryos generally and more clearly in the adults of Amphibia each cerebral hemisphere is naturally divided into four parts which correspond respectively with the four primary laminae of the lateral wall of the neural tube whose evagination produced the hemisphere. The relations of these parts of the telencephalon and diencephalon in adult Amphibia are shown in the diagrams of figs. 83 and 84 (see p. 477).

The olfactory bulb occupies the terminal part of the hemisphere (not of the *primary* neural axis) and the other four parts of the hemisphere are so related that the two ventral parts correspond with and pass backward directly into the ventral or motor lamina of the lower parts of the neural tube, the visceral efferent functions predominating in the ventro-median part and the somatic efferent in the ventro-lateral part. The two dorsal parts of the hemisphere correspond with the dorsal or sensory lamina of the neural tube, but direct continuity between the telencephalic and diencephalic segments of the dorsal lamina is interrupted in forms above the fishes by the great di-telencephalic fissure and (in Amniota) the posterior chorioidal fold.

The olfactory bulb was undoubtedly the site of the initial telencephalic evagination, but afterwards secondary olfactory tissue and correlation tissue of all four laminae of the rostral end of the neural tube were involved in this evagination and then further differentiated *in situ*.

Primitively the evaginated cerebral hemisphere was simply a primary and secondary olfactory center. In very early phylogenetic stages ascending fibers entered this secondary olfactory center from the pars dorsalis thalami for olfacto-tactile correlation, etc., and from the hypothalamus for olfacto-visceral correlations; and as we ascend the phylogenetic series this non-olfactory correlation tissue assumes relatively greater importance. So far as this tissue serves simple stereotyped reflexes it is developed in the ventral part of the hemisphere—the visceral centers medially and the somatic centers laterally. The olfactory component of the latter center plays progressively a smaller part in higher animals until this tissue becomes the true corpus striatum. While the ventral parts of the hemisphere are therefore favorably situated to serve simple, rapid stereotyped reflexes, the dorsal parts of the hemisphere (pars pallialis, Gaupp,) not being in direct connection with the corresponding diencephalic and lower regions of the brain in forms above fishes, are not well adapted for these rapid responses but rather for the slower and more complex discriminative reactions and (in higher animals) intelligent acts. Thus the two dorsal parts are in the course of the phylogeny gradually transformed from secondary olfactory nuclei into true cortex cerebri. Both dorsal parts continue throughout the phylogeny to receive some olfactory fibers, but these are much more numerous in the dorso-lateral part, which forms the pyriform lobe. Accordingly the latter in all mammals is more like the primordial secondary olfactory tissue than are the other parts of the pars pallialis.

Since the dorso-medial part of the hemisphere is to a less extent under the direct domination of any single one of the functional systems which enter into the cerebral hemisphere, in it the higher correlation tissue was first developed. The preponderating element at first in this pallial correlating apparatus was un-

doubtedly olfaction. Nevertheless cerebral cortex is not developed under the influence of any single sensory system, no matter how elaborately organized, and it is probable that the primordium hippocampi, even in selachians and amphibians, is concerned with the correlation of all of the various types of afferent impulses which reach the cerebral hemisphere in these animals.

These considerations permit the formulation of a provisional definition of cerebral cortex (p. 491) and suggest some modifications of the BNA subdivision of the prosencephalon (p. 494).

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REFERENCE LETTERS

a.d.—angulus dorsalis of hemisphere, separating pars dorso-medialis from pars dorso-lateralis
a.v.—angulus ventralis of hemisphere, separating pars ventromedialis from pars ventro-lateralis
bl—blood sinus
b.olf. and *bulbus olf.*—bulbus olfactorius
c.a.—commissura anterior
c.a.lat.—decussation of lateral forebrain tract in anterior commissure
c.a.med.—decussation of medial forebrain tract in anterior commissure
c.d.—commissura dorsalis (com. hippocampi)
c.d.m.—cortex dorso-medialis
c.dors.—cortex dorsalis
c.f.—columna fornicis
c.gen.lat.—corpus geniculatum laterale
c.hip.—commissura hippocampi
c.l.—cortex lateralis
c.olf.d.—dorsal olfactory commissure
com.po.—commissura postoptica
com.post.—commissura posterior
com.sup.—commissura superior
c.p.a.—commissura pallii anterior
c.p.p.—commissura pallii posterior
c.v.—commissura ventralis (com. anterior)
d.m.r.—dorso-medial ridge
dorsal assoc. tr.—dorsal association tract
d.s.—dorsal sac (post-velar arch of Minot)
e.m.—eminentia medialis, Kupffer (nucleus lateralis septi)
em.po.—eminentia postolfactoria
em.thal.—eminentia thalami
ep.—epiphysis
F.—interventricular foramen
f.a.—fissura arcuata
f.ch.—fissura chorioidea, Kupffer (fissura limitans hippocampi)
f.d.—fascia dentata
f.e.—fissura endorhinalis
f.i.—sulcus marking the position of the foramen interventriculare
fim.—fimbria
f.l.—fissura limitans hippocampi
f.retrofl.—fasciculus retroflexus
g.c.—granule cell layer of olfactory bulb
gl.—olfactory glomeruli
hab.—habenula
hip.—hippocampus
hyp.—hypophysis
hyth.—hypothalamus
lat.f.b.t.—lateral forebrain tract
l.t.—lamina terminalis
m—corpus mediale, Kupffer (part of primordium hippocampi)
m[1].—corpus precommissurale, Kupffer
m.c.—mitral cell layer of olfactory bulb
med.f.b.t.—medial forebrain tract
m.f.—medial fiber tract, Kupffer
mol.—molecular layer of olfactory bulb
n.ac.s.—nucleus accumbens septi
n.c.h.—nucleus of commissura hippocampi and nucleus of tractus cortico-habenularis
n.c.p.—nucleus of commissura pallii posterior
n.l.—nucleus lateralis septi
n.med.s.—nucleus medianus septi
n.ol.—nervus olfactorius
m.olf.ant.—nucleus olfactorius anterior
n.olf.v.m.—ventro-medial olfactory nucleus
n.op.—nervus opticus
n.po.—nucleus preopticus
n.po.pars ant.—nucleus preopticus, pars anterior
n.po.pars m.—nucleus preopticus, pars magnocellularis
n.term.—nervus terminalis
O.—locus in the primordial neural tube from which the olfactory bulb evaginates
op.ch.—optic chiasma

P.—paraphysis
pa.—pallium
para.—corpus paraterminale
pars d.l.—pars dorso-lateralis hemisphaerii
pars dors. thal.—pars dorsalis thalami
pars ven.thal.—pars ventralis thalami
pars v.l.—pars ventro-lateralis hemisphaerii
p.f.s.—pars fimbrialis septi
p.g.—periglomerular cells of olfactory bulb
pl.—plexus chorioideus of third ventricle
p.lat.—plexus lateralis
p.m.t.—plexus medius telencephali
prim.hip.—primordium hippocampi
R.—locus in the primordial neural tube from which the retinal epithelium evaginates
rec.n.—recessus neuroporicus
rec.s. and *rec.sup.*—recessus superior
r.po.—recessus preopticus
s.—septum
sac.d.—dorsal sac
s.d.—sulcus diencephalicus dorsalis
s.d.t.—telencephalic extension of diencephalicus sulcus dorsalis
sec.olf.—secondary olfactory nucleus
s.epen.—septum ependymale
s.ie.ant.—sulcus interencephalicus anterior, Kupffer
s.m.—sulcus diencephalicus medius
s.shab.—sulcus subhabenularis
st.—corpus striatum
str.med.—stria medullaris
s.v.—sulcus diencephalicus ventralis
t.—terminal ridge (ventral lip of blastopore, Johnston)
taenia thal. et forn.—union of taenia thalami with taenia fornicis
tectum mes.—tectum mesencephali
t.f.—taenia fornicis
tr.—torus transversus, Kupffer (ventral part of precommissural body)
tr.c.hab.—tractus cortico-habenularis
tr.c.hab.lat.—tractus cortico-habenularis lateralis
tr.hab.st.—tractus habenulo-striaticus
tr.hab.thal.—tractus habenulo-thalamicus.
tr.o.—medial and lateral forebrain tract with part of tractus olfactorius
tr.olf.—tractus olfactorius
tr.olf.d.lat.—tractus olfactorius dorsolateralis
tr.olf hab.—tractus olfacto-habenularis
tr.olf. hab. lat.—tractus olfacto-habenularis lateralis
tr.olf.hab.med.—tractus olfacto-habenularis medialis
tr.olf.lat.—tractus olfactorius lateralis
tr.olf.med.—tractus olfactorius medialis
tr.olf.v.lat.—tractus olfactorius ventrolateralis
tr.op.—tractus opticus
t.th.—taenia thalami
tub.olf.—tuberculum olfactorium
v.—velum transversum
ventral assoc. r.—ventral association tract
vl. and *vs.*—ventriculus lateralis
y.—rudiment of plexus lateralis
z.—transitory posterior chorioidal fold
z.lim.lat.—zona limitans lateralis
z.lim.med.—zona limitans medialis

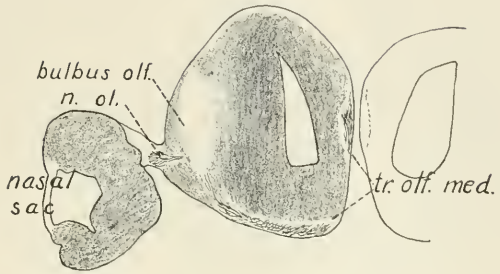
EXPLANATION OF FIGURES

1 to 5. A series of transverse sections through the brain of a specimen of Amblystoma tigrinum 17 mm. long (about 35 days after fertilization). Method of Ramón y Cajal. × 74.

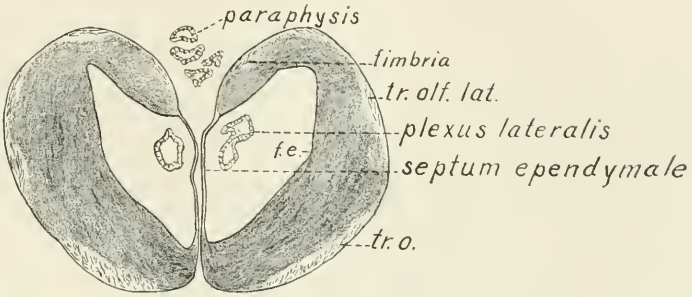
1. Through the olfactory bulb and illustrating the course of the nervus olfactorius and the division of the tractus olfactorius medialis into dorsal and ventral portions.

2. A short distance rostral to the interventricular foramen. The locus of the fissura endorhinalis is indicated by an ependymal groove at *f.e.* The dorsal division of the lateral olfactory tract (tr. olf. lat. dorsalis) characterizes the dorsolateral part of the hemisphere. *tr. o.* is a mixed system containing the medial and lateral components of the basal forebrain bundle and the ventral divisions of the medial and lateral olfactory tracts.

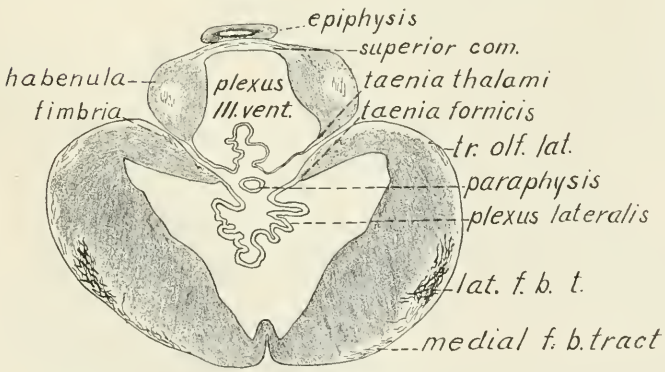
3. Through the interventricular foramen near its rostral end.


bulbus olf.
n. ol.
nasal sac
tr. olf. med.

1


paraphysis
fimbria
tr. olf. lat.
plexus lateralis
f.e.
septum ependymale
tr. o.

2


epiphysis
superior com.
habenula
plexus
taenia thalami
fimbria
III. vent.
taenia fornicis
tr. olf. lat.
paraphysis
plexus lateralis
lat. f. b. t.
medial f. b. tract

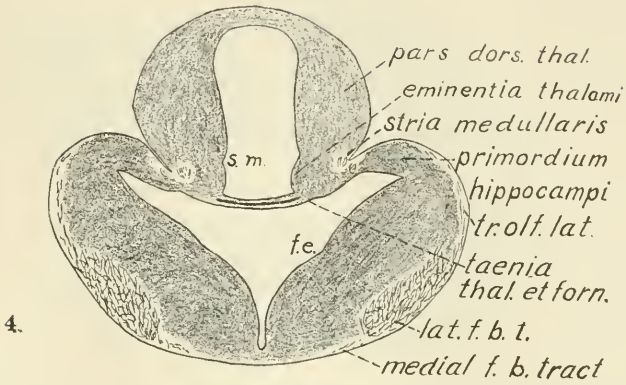
3

EXPLANATION OF FIGURES

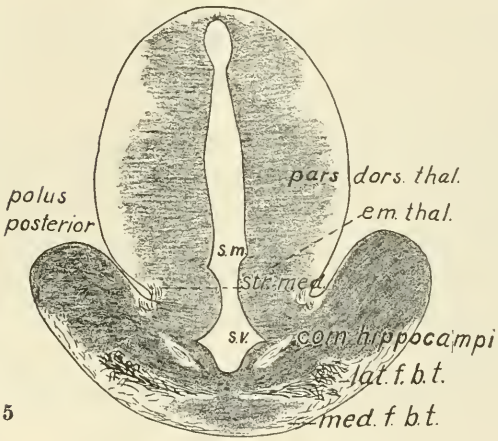
4. Through the caudal part of the interventricular foramen. At the union of the taenia thalami with the taenia fornicis (*taenia thal. et forn.*) the velum transversum stretches across between the third ventricle above and the ventriculus medius telencephali below. The fimbria complex of fig. 3 here separates into two parts, one of which enters the stria medullaris, the other passes caudad and ventrad to enter the commissura pallii anterior (fig. 5). The basal forebrain bundle is imperfectly separated into lateral and medial forebrain tracts.

5. Through the posterior pole and anterior commissure. The latter contains two distinct components, the ventral one a decussation of the medial forebrain tract (*med. f. b. t.*) and the dorsal one a decussation of the lateral forebrain tract (*lat. f. b. t.*). Dorsally of both of these is the very small commissura pallii anterior or commissura hippocampi.

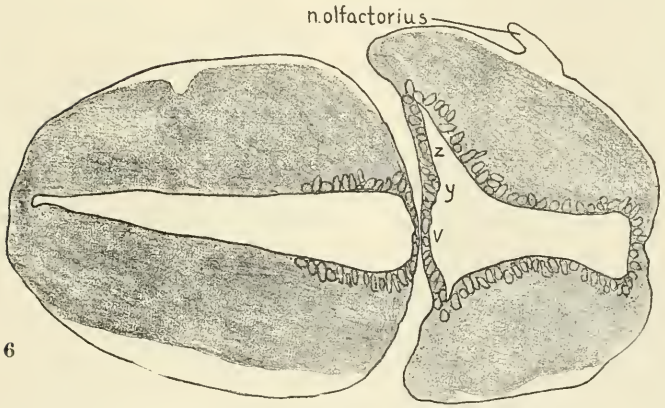
6. Section through the brain of a larva of Amblystoma tigrinum 10 mm. long (about 15 days after fertilization) so oriented as to pass through the region of the velum transversum (*v*) in approximately the horizontal plane, the right side being a little farther ventral than the left. × 104. The transitory posterior chorioidal fold is seen at *z* and the first rudiment of the plexus lateralis at *y*.


pars dors. thal.
eminentia thalami
stria medullaris
primordium
hippocampi
tr. olf. lat.
taenia
thal. et forn.
lat. f. b. t.
medial f. b. tract
S. m.
f. e.

4.


pars dors. thal.
em. thal.
polus posterior
s. m.
Str. med.
s. v.
corn. hippocampi
lat. f. b. t.
med. f. b. t.

5


n. olfactorius
z
y
v

6

EXPLANATION OF FIGURES

7. A section from the same embryo taken 20 micra farther ventrad. The extreme dorsal border of the eminentia thalami is seen on the right side.

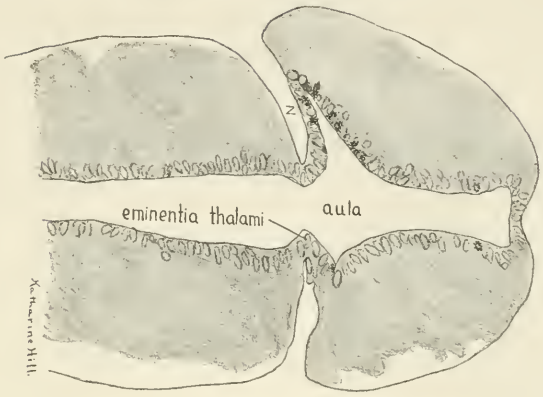
8 to 21. A series of transverse sections through the brain of adult Amblystoma tigrinum, stained by Weigert's method. × 20.

8. Section through the rostral end of the olfactory bulbs. The granular layers of the two bulbs come into contact in the medial plane. This is the site of the interbulbar union seen in Anura, though in Urodela the fusion does not take place.

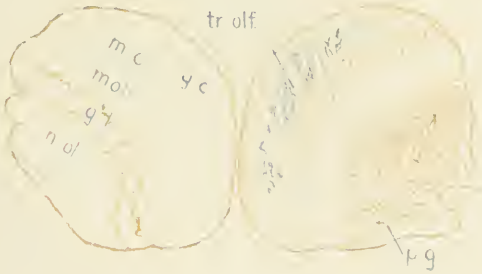
9. Section .25 mm. farther caudad passing through the extreme rostral tip of the lateral ventricle and nucleus olfactorius anterior.

10. Section .5 mm. caudad of fig. 9, illustrating the expansion of the nucleus olfactorius anterior.

11. Section .66 mm. caudad of the last, through the caudal end of the olfactory bulb and the rostral end of the primordium hippocampi.

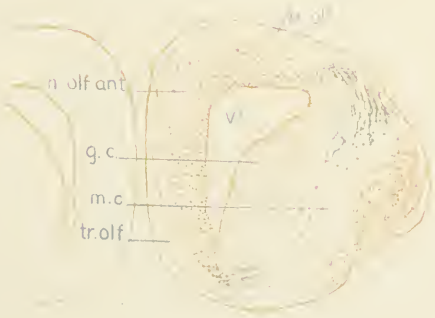

z
eminentia thalami
aula

7

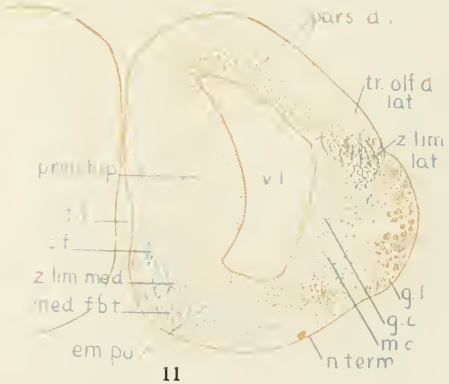

tr olf
m c
y c
p g

8

9


n olf ant
g. c
m.c
tr.olf

10


pars a.
tr. olf a
lat
z lim
lat
v l
z lim med
em pu
g l
g c
m c
n term

11

EXPLANATION OF FIGURES

12. Section .8 mm. farther caudad through the middle of the septum.

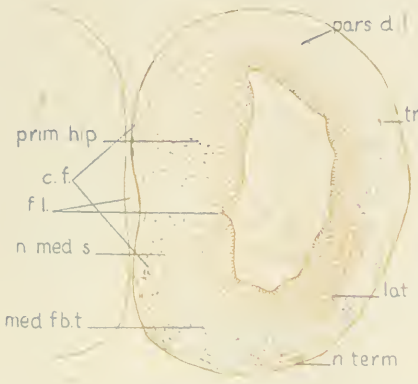
13. Section .3 mm. farther caudad.

14. Section immediately rostral to the interventricular foramen, through the septum ependymale.

15. Section through the interventricular foramen.

16. Section through the rostral part of the anterior commissure ridge immediately caudal to the interventricular foramen.

17. Section through the caudal part of the anterior commissure ridge.

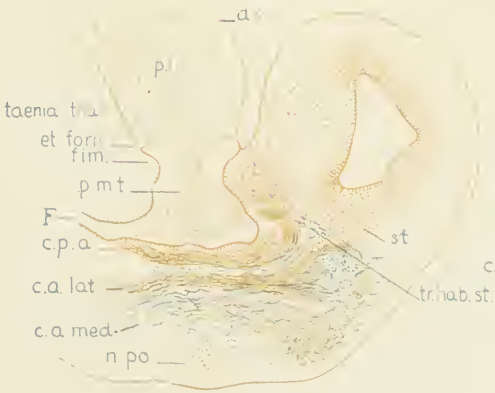


12

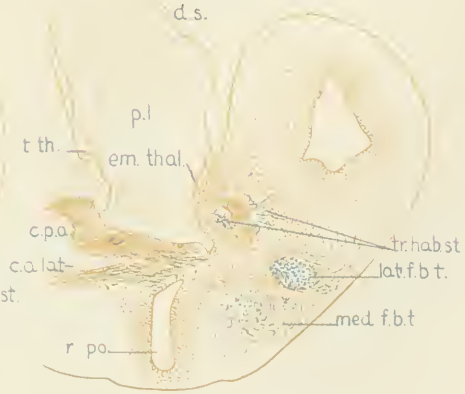
13

14

15



16



17

EXPLANATION OF FIGURES

18. Section through the rostral end of the thalamus. The pars ventralis thalami is bounded above by the sulcus diencephalicus medius (*s.m.*) and below by the sulcus diencephalicus ventralis (*s.v.*). A slight sulcus separates its dorsal portion from the ventral, the dorsal portion being the eminentia thalami, which gives rise to the nucleus of the commissura hippocampi of Anura.

19. Section taken 144 micra farther caudad than fig. 18.

20. Section through the middle of thalamus.

21. Section through the caudal part of the thalamus and the rostral end of the mesencephalon.

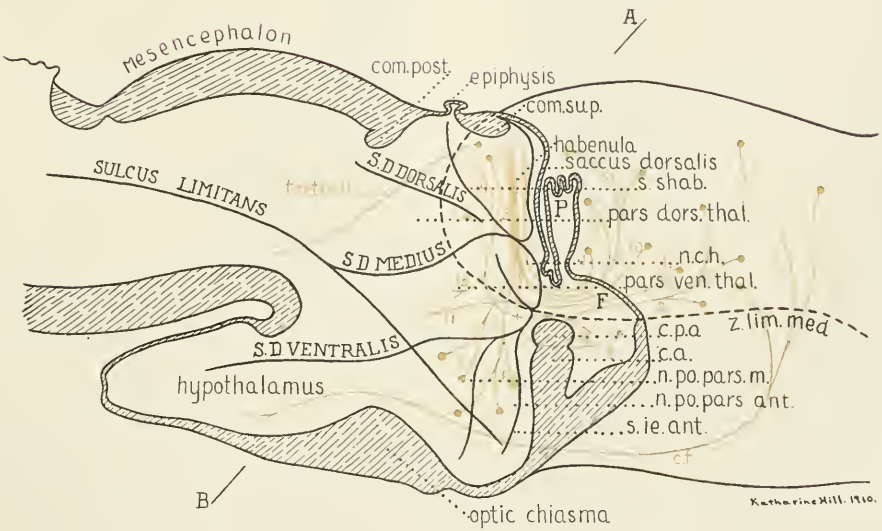
22. Diagram of the relations of the diencephalic ependymal sulci and components of the stria medullaris (in brown) in adult Amblystoma tigrinum. The outlines of the medial sagittal section and of the diencephalic sulci are based on a graphic reconstruction from sagittal sections (× 20). Cf. figs. 8 to 21, drawn to the same scale from transverse sections. The numbers given to the fiber tracts refer to the paragraphs on pages 427 ff. of the text. The decussation of part of the stria medullaris is not indicated. The line *A-B* indicates the plane of the section shown in fig. 83.

18

19

20

21


A
mesencephalon
com.post.
epiphysis
com.sup.
habenula
saccus dorsalis
S.D DORSALIS
s. hab.
SULCUS LIMITANS
P
pars dors. thal.
S.D MEDIUS
n.c.h.
pars ven. thal.
F
c.p.a
z. lim. med
S.D VENTRALIS
c.a.
hypothalamus
n. po. pars. m.
n. po. pars ant.
s. ie. ant.
B
optic chiasma
Katharine Hill. 1910.

22

EXPLANATION OF FIGURES

23. Transverse section through the brain of Amphiuma in front of the lamina terminalis. After Osborn ('83). × 8.

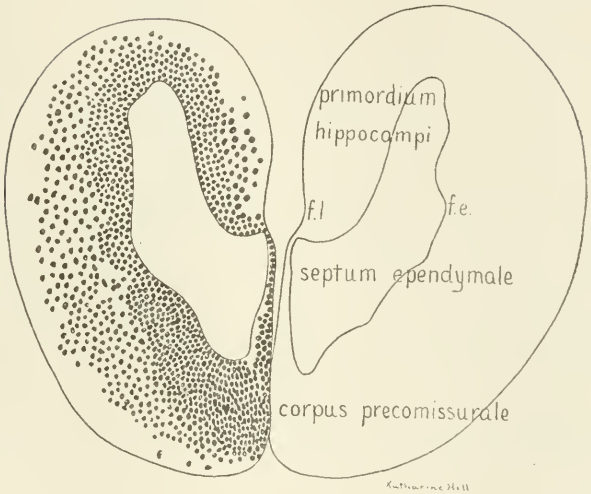
24. Transverse section through the brain of a specimen of Amblystoma larva 35 mm. long, stained with haematoxylin. × 74.

The section passes immediately in front of the interventricular foramen, showing the nucleus medianus septi within the corpus precommissurale invading the septum ependymale.

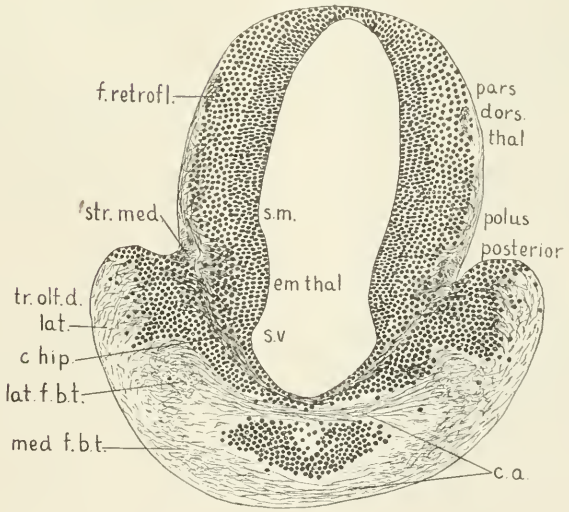
25. Transverse section through the brain of a specimen of Amblystoma tigrinum larva 32 mm. long, stained with alum cochineal and Lyon blue. × 50.

The commissura anterior (*c.a.*) is composed chiefly of decussating fibers from the lateral and medial forebrain tracts.

23


primordium
hippocampi
f.l
f.e.
septum ependymale
corpus precomissurale

24


f. retrofl.
pars
dors.
thal
str. med.
s.m.
polus
posterior
em thal
tr. olf. d.
lat.
s.v
c hip.
lat. f. b. t.
med. f. b. t.
c. a.

25

EXPLANATION OF FIGURES

26. Section from the same series as fig. 25, 75 micra farther caudad.

27. Transverse section through the brain of a 29.6 mm. larva of Necturus maculatus. Drawn from a preparation in the Harvard Embryological Collection (series 536, section 80). × 28.

The right side is farther caudad than the left. The nucleus medianus septi (*s* on the left) extends backward into the dorsal wall of the septum ependymal to form a pars fimbrialis septi (*p. f. s.* on the right); cf. fig. 23. The fissura limitans hippocampi (*f.l.*) separates the septum from the primordium hippocampi above.

28. Section taken 28 micra caudad of fig. 27 through the septum ependymale (*s. epen.*). The septum is interrupted by the interventricular foramen in the next section caudad. (Harvard Embryological Collection, series 536, section 82).

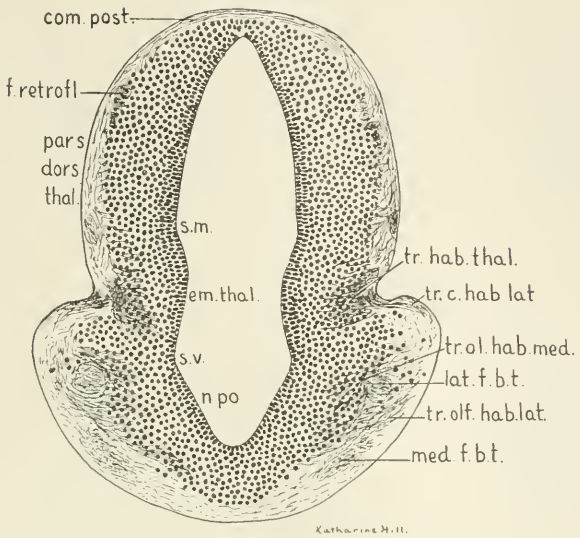
29. Transverse section through the brain of an old frog tadpole, approaching the metamorphosis, taken in front of the lamina terminalis. Stained with haematoxylin and acid fuchsin. × 30.

30, 31, 32. Three sections through the brain of another frog tadpole of the same age as the last. Haematoxylin preparations. × 30.

30. Through the lamina terminalis immediately rostral to the interventricular foramen. The nucleus medianus septi (precommissural body) is very large and extends dorsally of the level of the foramen.

31. Through the interventricular foramen. The small portion of the precommissural body above the foramen is the pars fimbrialis septi (Kappers).

32. Through the anterior commissure. The section is somewhat oblique with the left side farther caudad. On this side the rostral end of the nucleus of the hippocampal commissure (*n. c. h.*) is shown.



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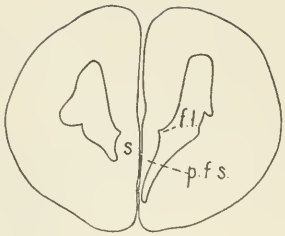

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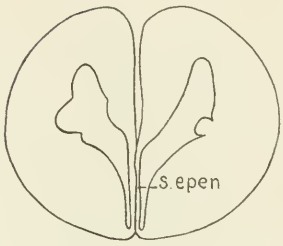

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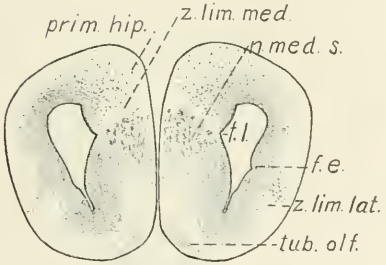

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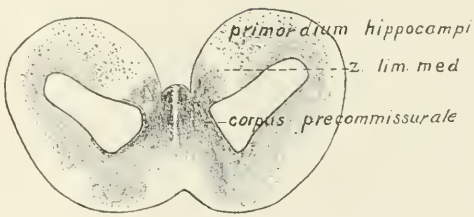

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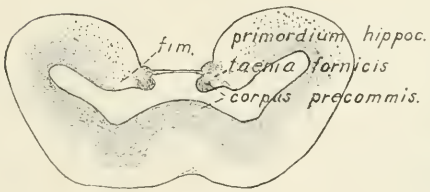

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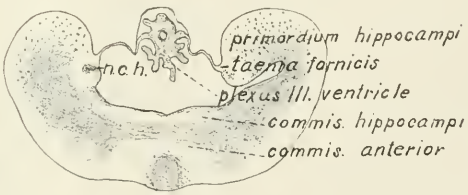


32

EXPLANATION OF FIGURES

33 to 39. Transverse sections through the brain of the same specimen shown in fig. 29. × 30.

33. Through the posterior poles of the hemispheres. The nucleus of the commissura hippocampi (*n. c. h.*) is the rostral end of the pars ventralis thalami.

34. Through the rostral border of the habenula and superior commissure. The cells marked *striatum* are in close relation with those of the pars magnocellularis of the preoptic nucleus. They extend forward into the pars ventro-lateralis of the hemisphere and represent a portion of the primordium of the corpus striatum which has not been evaginated into the cerebral hemisphere.

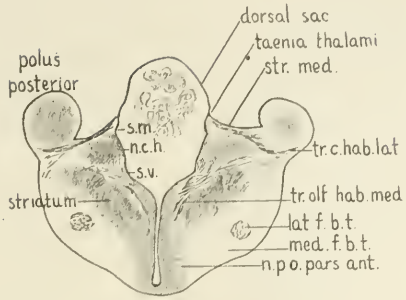
35. Three sections (60 micra) farther back. Note the very large size of the preoptic nucleus and the moderate development of the pars ventralis thalami. The rostral tip of the pars dorsalis thalami and corpus geniculatum laterale are shown.

36. Through the caudal end of the habenula.

37. Through the optic chiasma.

38. Through the post-optic commissure.

39. Through the rostral end of the posterior commissure and hypothalamus.



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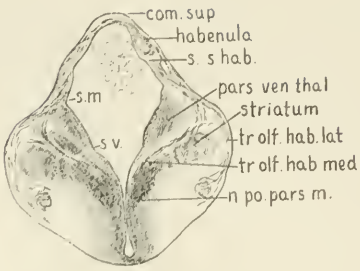
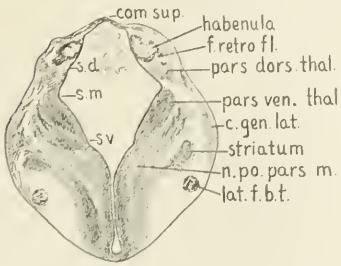
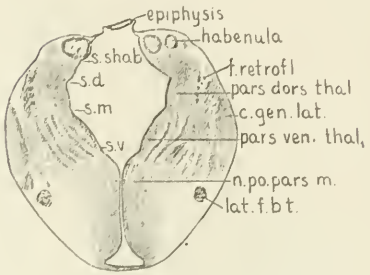


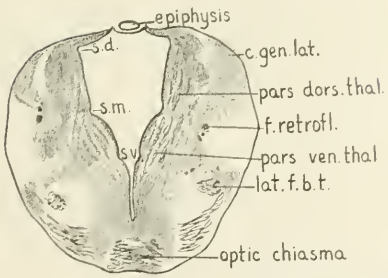
34



35



36



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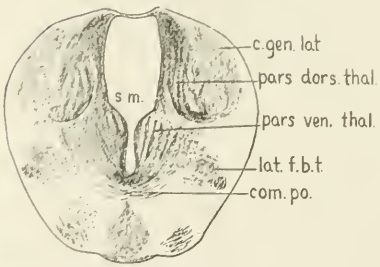

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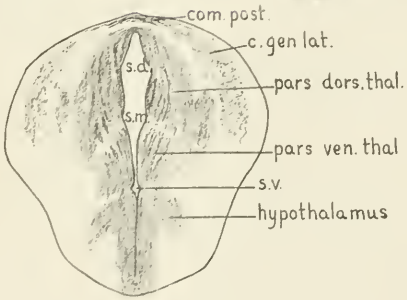
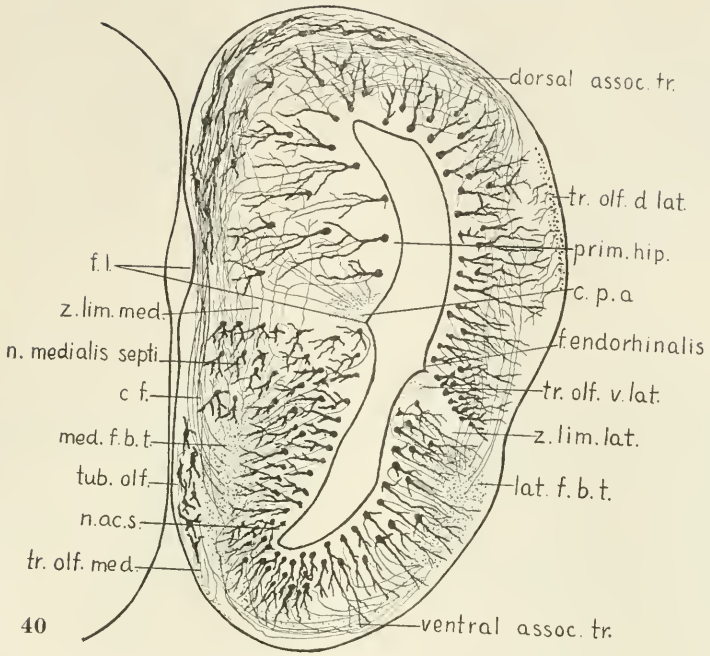


39

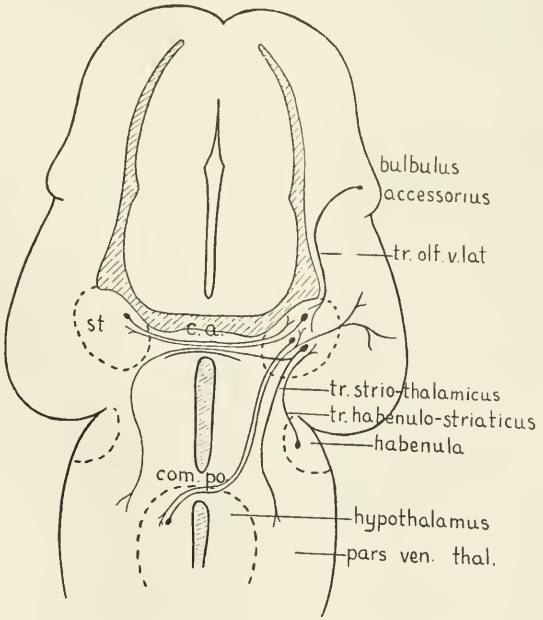
EXPLANATION OF FIGURES

40. A diagrammatic cross section of the cerebral hemisphere of the frog taken midway between the lamina terminalis and the olfactory bulb. It illustrates the four parts of the hemisphere, separated by the dorsal and ventral angles and the median and lateral limiting zones; also the dorsal and ventral association tracts and some of the tracts which pass across the limiting zones.

41. Diagram of a horizontal section of the brain of the frog to illustrate the connections of the bulbulus accessorius, ventro-lateral olfactory tract and so-called corpus striatum.



40



41

Katherine Hill 1910

EXPLANATION OF FIGURES

42. Transverse section through the middle of the cerebral hemisphere of Lacert muralis (?) embryo of 7.6 mm. (measured across the greatest diameter of the coil as it lies in the egg). Harvard Embryological Collection, series 1601, section 121. × 24.

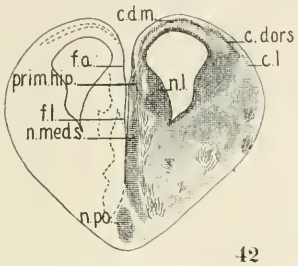
43, 44, 45. Three transverse sections through the cerebral hemisphere of a 16.7 mm. embryo of Chrysemys marginata. Harvard Embryological Collection, series 1092. × 28.

43. Section 175, through the middle of the septum.

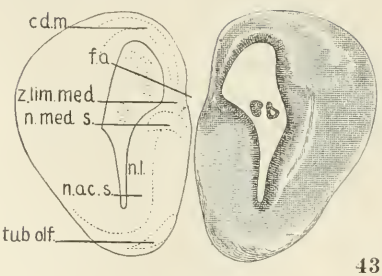
44. Section 190, taken 150 micra farther caudad than the last, showing an undifferentiated remnant of the primordium hippocampi ventrally of the cortex hippocampi.

45. Section 195, taken 50 micra farther caudad, immediately rostral to the interventricular foramen, illustrating the septum ependymale.

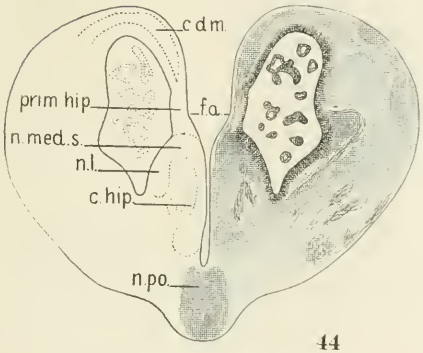
46. Section taken in the same plane as fig. 45 through an older embryo of Chrysemys marginata of 27 mm. Harvard Embryological Collection, series 1096, section 168. × 36.


c.d.m.
c.dors
c.l
f.a.
prim.hip.
n.l.
f.l.
n.med.s.
n.po.

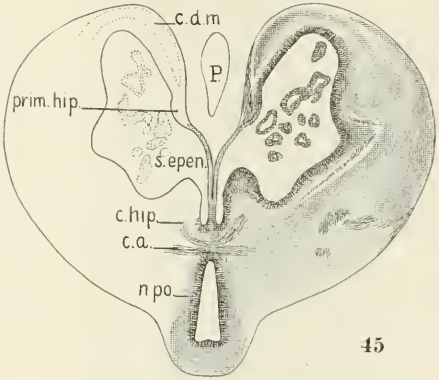
42


c.d.m.
f.a.
z.lim.med.
n.med. s.
n.l.
n.ac.s.
tub olf.

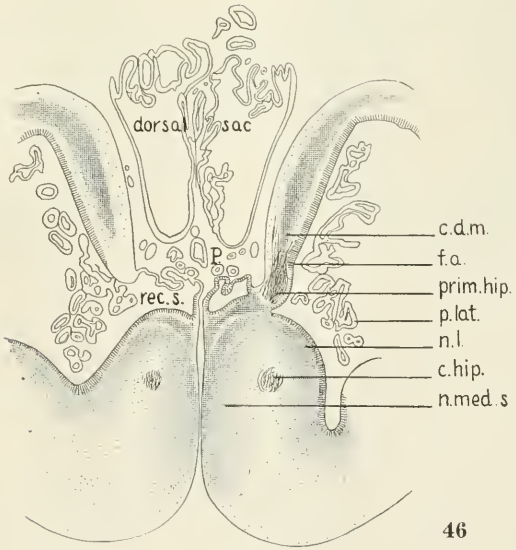
43


c.d.m.
prim hip
f.a.
n.med..s.
n.l.
c.hip
n.po.

44


c.d.m.
P.
prim.hip.
s.epen.
c.hip.
c.a.
n.po.

45


dorsal sac
P.
rec.s.
c.d.m.
f.a.
prim.hip.
p.lat.
n.l.
c.hip.
n.med.s

46

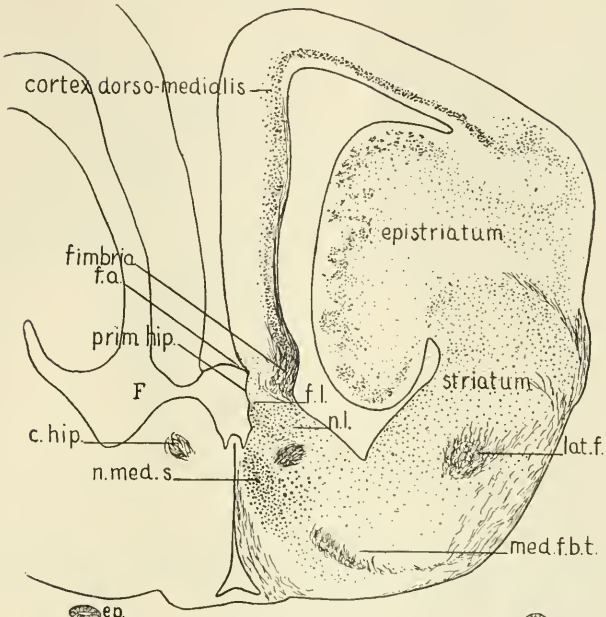
EXPLANATION OF FIGURES

47. Transverse section through the brain of the box tortoise, Cistudo carolina. × 15. The section passes through the interventricular foramen on the left side and immediately rostral to it on the right.

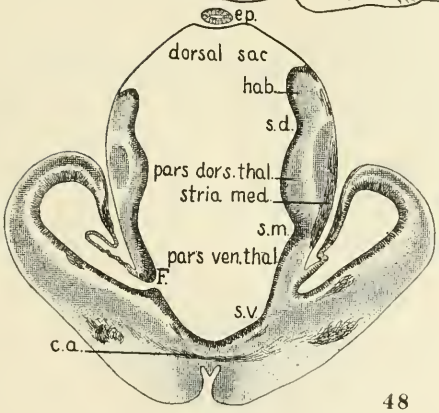
48. Section through the brain of a 12.3 mm. embryo of Chrysemys marginata cut transversely to the sagittal plane and so inclined that the dorsal surface lies farther caudad than the ventral. Harvard Embryological Collection, series 1087, section 87. × 28.

49. Section 96 of the same series as fig. 48. These two sections illustrate the subdivisions of the diencephalon.

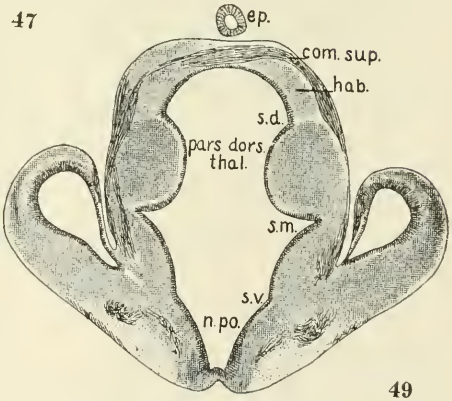
50. Sagittal section through the brain of a 26.7 mm. embryo of Chrysemys marginata. Harvard Embryological Collection, series 1097, section 413. × 15.


cortex dorso-medialis
epistriatum
fimbria
f.a.
prim. hip.
F
f.l.
n.l.
striatum
c. hip.
n. med. s.
lat. f.b.t.
med. f.b.t.

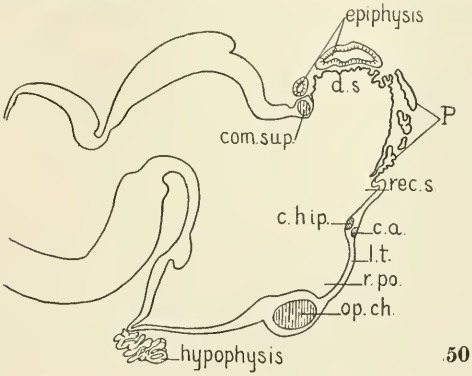
47


ep.
dorsal sac
hab.
s.d.
pars dors. thal.
stria med.
s.m.
pars ven. thal.
F.
s.v.
c.a.

48


ep.
com. sup.
hab.
s.d.
pars dors. thal.
s.m.
s.v.
n. po.

49


epiphysis
d. s
P
com. sup.
rec. s
c. hip.
c.a.
l.t.
r. po.
op. ch.
hypophysis

50

EXPLANATION OF FIGURES

51. Section 402 from the same series as fig. 50, taken 11 sections from the sagtital plane . × 15.

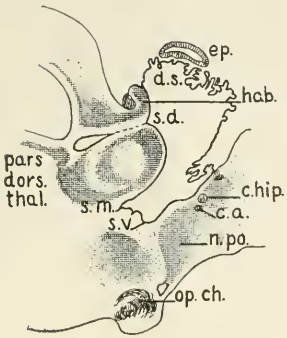
52. A parasagittal section through the brain of a specimen of Lacerta vivipara (?) with a total length when uncoiled of about 36 mm. (head 5 mm. long). Harvard Embryological Collection, series 603, section 201. × 24. On this figure is indicated the approximate planes of figs. 53 to 57.

53 to 57. A series of sections through the brain of a specimen of Lacerta vivipara (?) of about the same age as the one last figured. Harvard Embryological Collection, series 604. × 24. For planes of sections, see fig. 52.

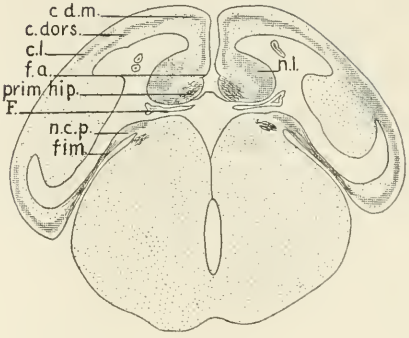
53. Section through the anterior commissure.

54. Section immediately ventrally of the interventricular foramen.

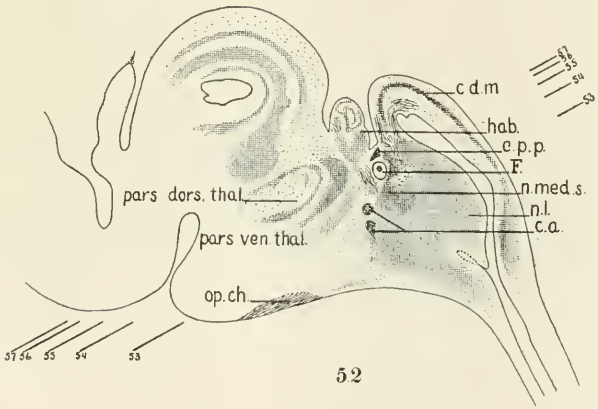
55. Section through the interventricular foramen.


ep.
d.s.
hab.
s.d.
pars
dors.
thal.
s.m.
s.v.
c.hip.
c.a.
n.po.
op.ch.

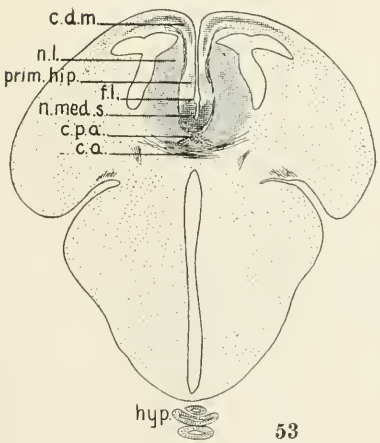
51


c.d.m.
c.dors.
c.l.
f.a.
prim.hip.
F.
n.c.p.
fim.
n.l.

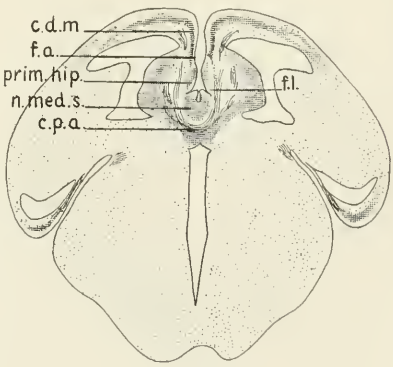
55


c.d.m
hab.
c.p.p.
F.
n.med.s.
n.l.
c.a.
pars dors. thal.
pars ven. thal.
op.ch
57 56 55 54 53

52


c.d.m
n.l.
prim.hip.
f.l.
n.med.s.
c.p.a.
c.a.
hyp.

53


c.d.m
f.a.
prim.hip.
n.med.s.
c.p.a.
f.l.

54

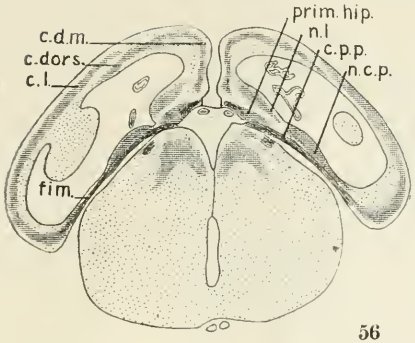
EXPLANATION OF FIGURES

56. Section through the fimbria and nucleus of the commissura pallii posterior.

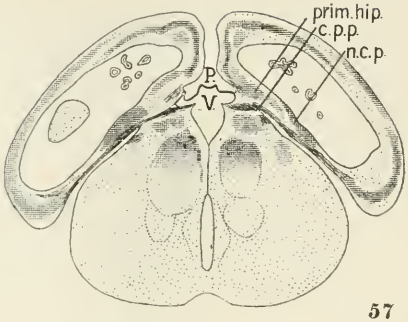
57. Section through the commissura pallii posterior.

58. Horizontal section through the commissura pallii posterior of a 25.2 mm. embryo of Sphenodon. Harvard Embryological Collection, series 1490, section 341. × 29.

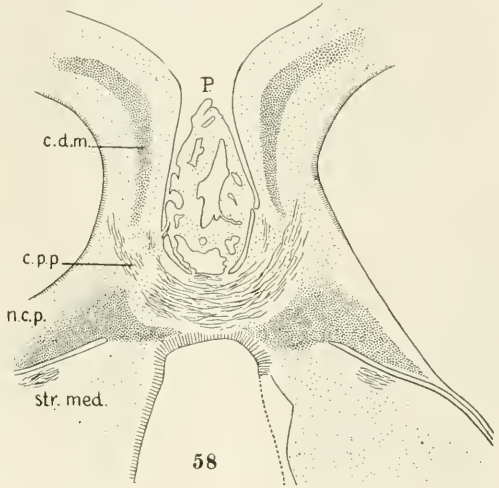
59. Section 347 of the same series as the last, taken 70 micra farther dorsally.


c.d.m.
c.dors.
c.l.
fim.
prim.hip.
n.l.
c.p.p.
n.c.p.

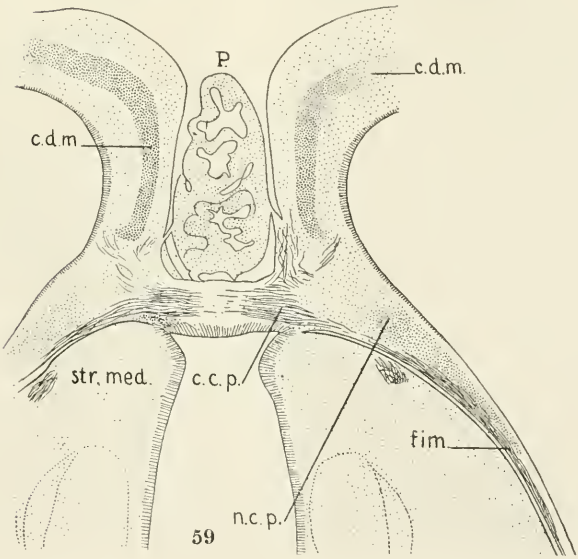
56


prim.hip.
c.p.p.
n.c.p.
P.
V.

57


P.
c.d.m.
c.p.p.
n.c.p.
str. med.

58

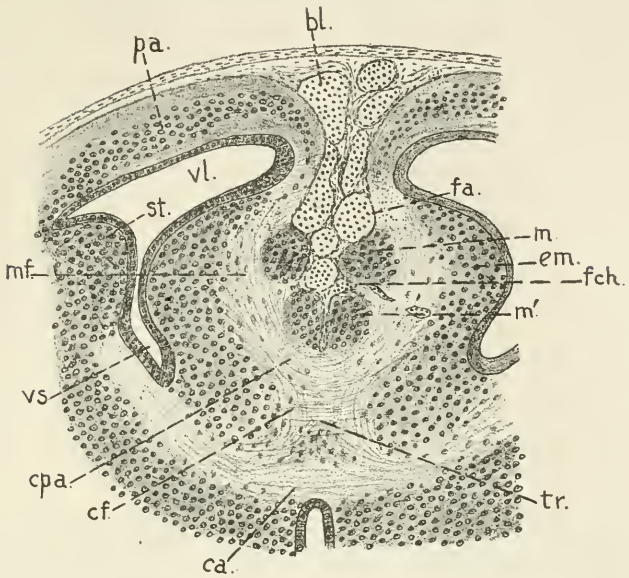

P.
c.d.m.
c.d.m.
str. med.
c.c.p.
fim.
n.c.p.

59

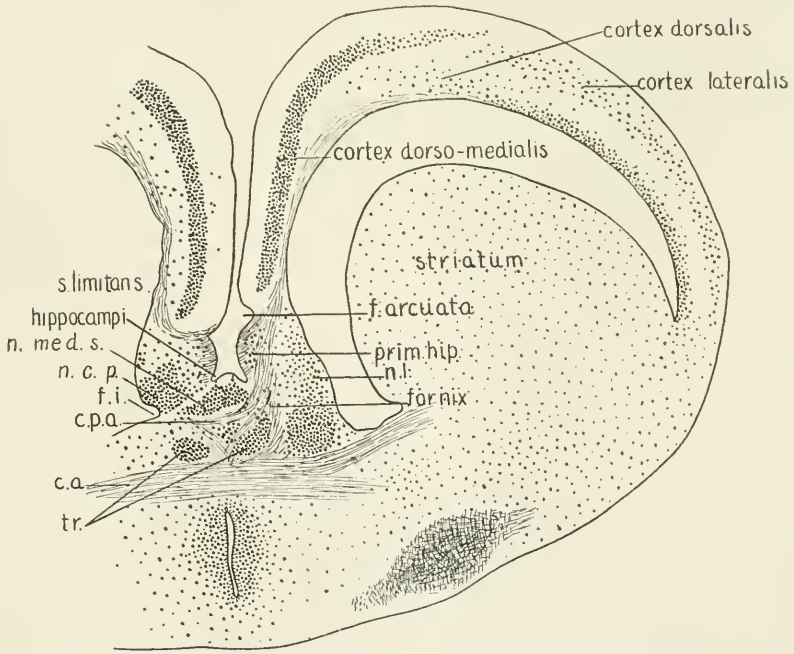
EXPLANATION OF FIGURES

60. Cross section through the lamina terminalis and precommissural body of an advanced larva of Anguis fragilis. × 75. After Kupffer ('06, p. 234). The reference letters are those of the original.

61. A transverse section through the brain of Phrynosoma cornutum (Harlan). Haematoxylin preparation. × 30. The section passes immediately rostral to the foramen interventriculare, the left side being slightly farther caudad.


pa.
bl.
vl.
st.
fa.
m.
em.
fch.
mf.
m'
vs
cpa.
cf.
tr.
ca.

60


cortex dorsalis
cortex lateralis
cortex dorso-medialis
striatum
s.limitans
f.arcuata
hippocampi
n. med. s.
prim.hip.
n. c. p.
n.l.
f. i.
fornix
c.p.a.
c.a.
tr.

61

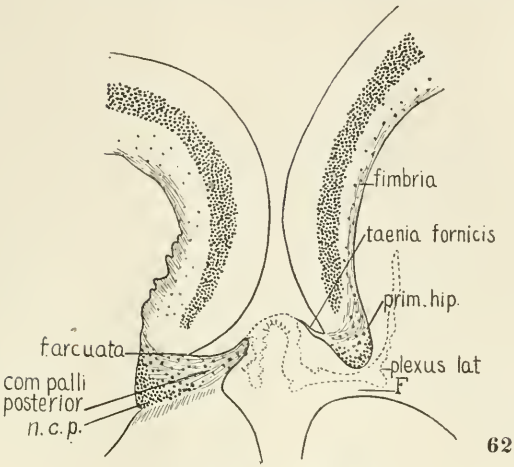
EXPLANATION OF FIGURES

62. A section from the same series farther caudad. x 30. On the right side it passes through the interventricular foramen, of the left immediately caudad of it.

63, 64, and 65. Three horizontal sections through the brain of the lizard, Sceloporus undulatus (Daudin). Haematoxylin preparation. The right side is slightly farther ventral than the left. × 20.

63. Through the dorsal part of the hemispheres.

64. On the right side the section passes through the interventricular foramen; on the left side above it; the precommissural body surrounds the foramen.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plexus lat
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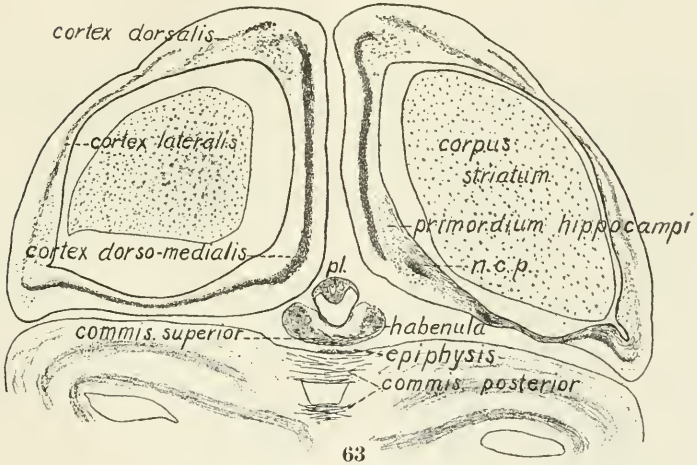
farcuata
com palli posterior
n. c. p.

62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cortex lateralis
corpus striatum
primordium hippocampi
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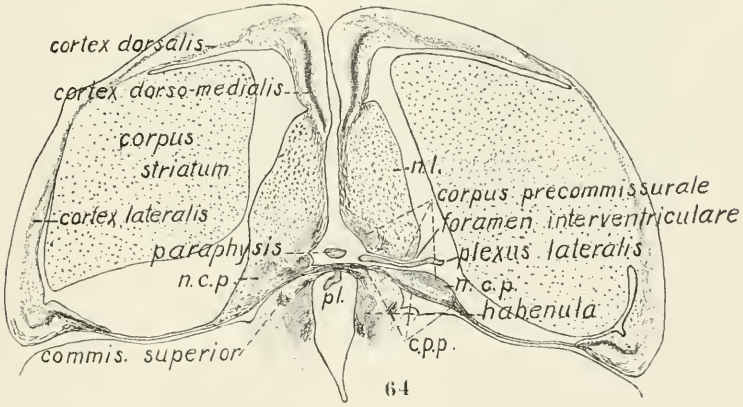
cortex dorso-medialis
pl.
commis. superior
habenula
epiphysis
commis. posterior

63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corpus striatum
cortex lateralis
paraphysis
n. c. p.
pl.
n. l.
corpus precommissurale
foramen interventriculare
plexus lateralis
n. c. p.
habenula
c. p. p.
commis. superior

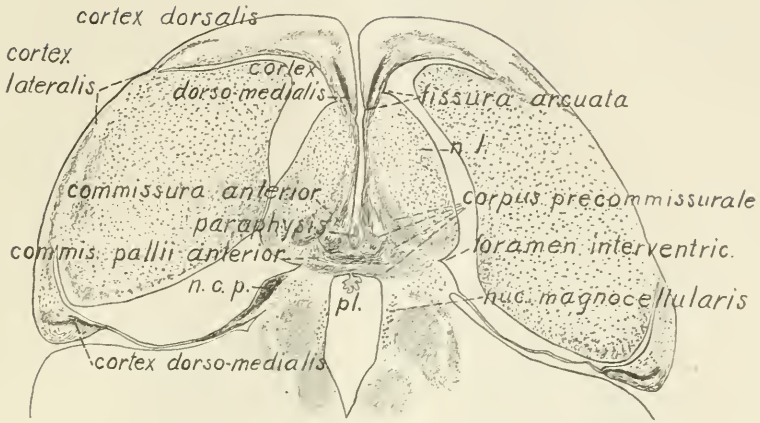
64

EXPLANATION OF FIGURES

65. Immediately ventral to the interventricular foramina, illustrating the way in which the precommissural body forms the "bed" of the commissura pallii anterior and commissura anterior, the latter crossing immediately ventrally of the point designated.

66 to 70. Sections from a transverse series through the brain of young Alligator mississippiensis about 25 cm. long, stained by the method of Ramón y Cajal. × 15.

66. Through the caudal part of the septum, illustrating its medial and lateral nuclei and their relations to the primordium hippocampi.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cortex dorso-medialis
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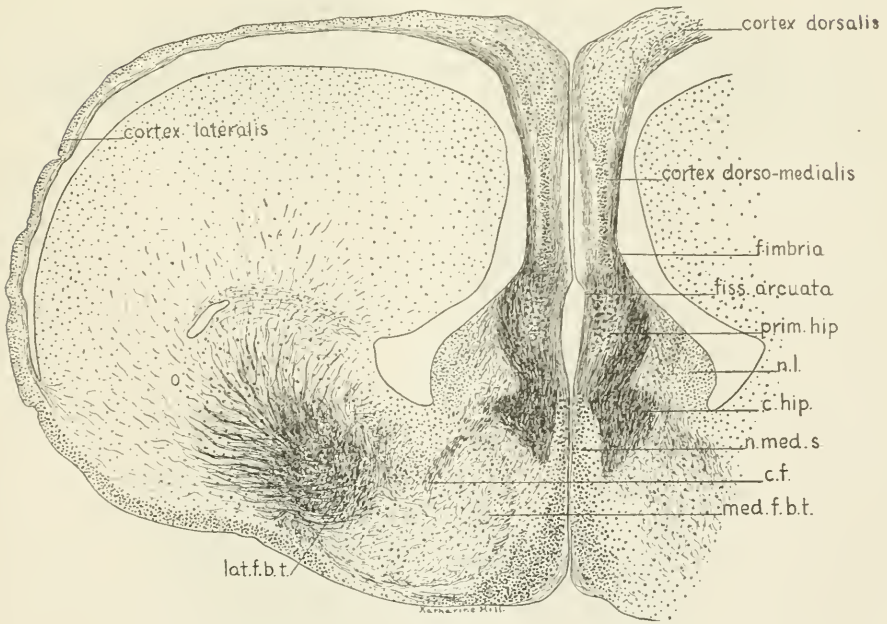
n. l.
commissura anterior
paraphysis
corpus precommissurale
commis. pallii anterior
foramen interventric.
n. c. p.
pl.
nuc. magnocellularis
cortex dorso-medialis

65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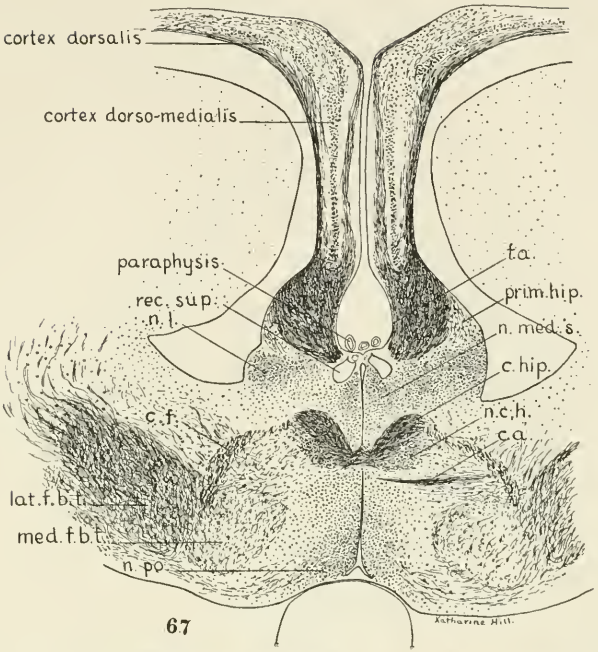
fiss. arcuata
prim. hip
n.l.
c. hip.
n. med. s
c.f.
med. f. b. t.
lat. f. b. t.

66

EXPLANATION OF FIGURES

67. Through the anterior commissure and commissura hippocampi.

68. Through the interventricular foramina. The nucleus of the commissura hippocampi (*n.c.h.*) is continuous with the nucleus of the tractus cortico-habenularis (*tr.c.hab.*)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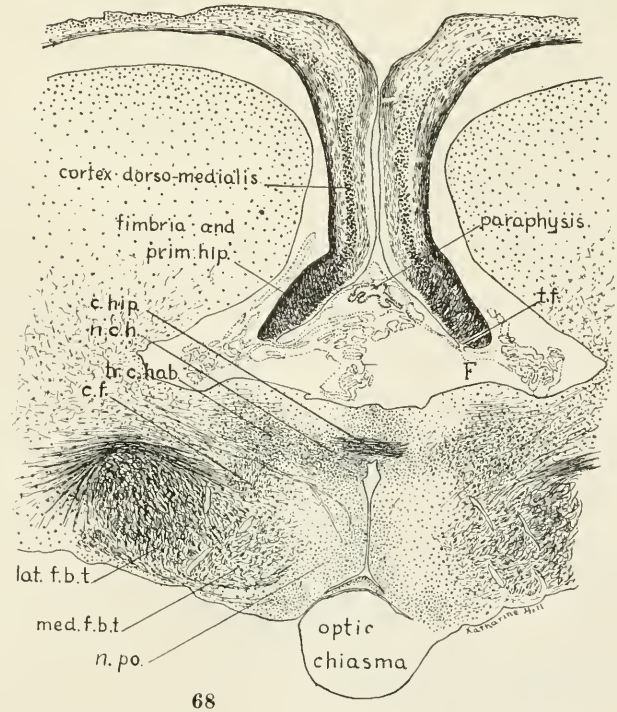
n. med. s.
c. hip.
c. f.
n.c.h.
c.a.
lat. f.b.t.
med. f.b.t.
n. po.
Katharine Hill.

67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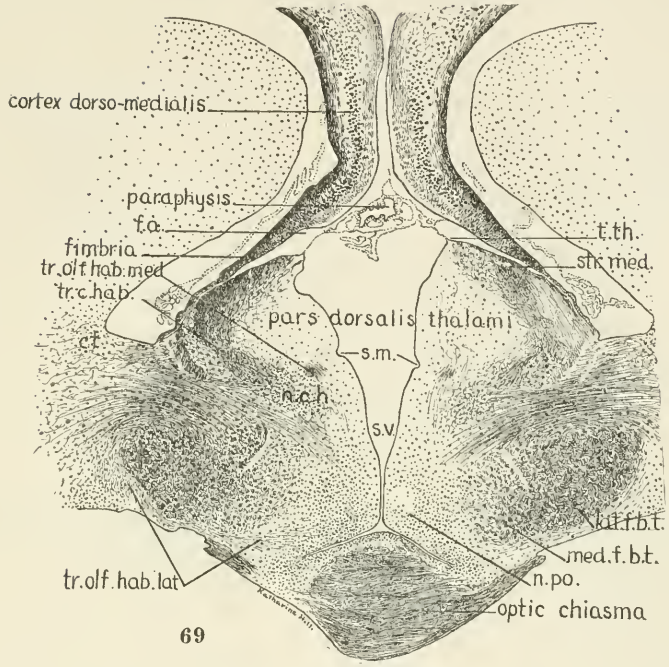
fimbria and
prim. hip.
paraphysis.
t.f.
c. hip.
n.c.h.
tr. c. hab.
c.f.
F
lat. f.b.t.
med. f.b.t.
n. po.
optic
chiasma
Katharine Hill.

68

EXPLANATION OF FIGURES

69. Through the rostral end of the diencephalon.

70. Through the middle of the diencephalon, including the rostral end of the massa intermedia.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paraphysis
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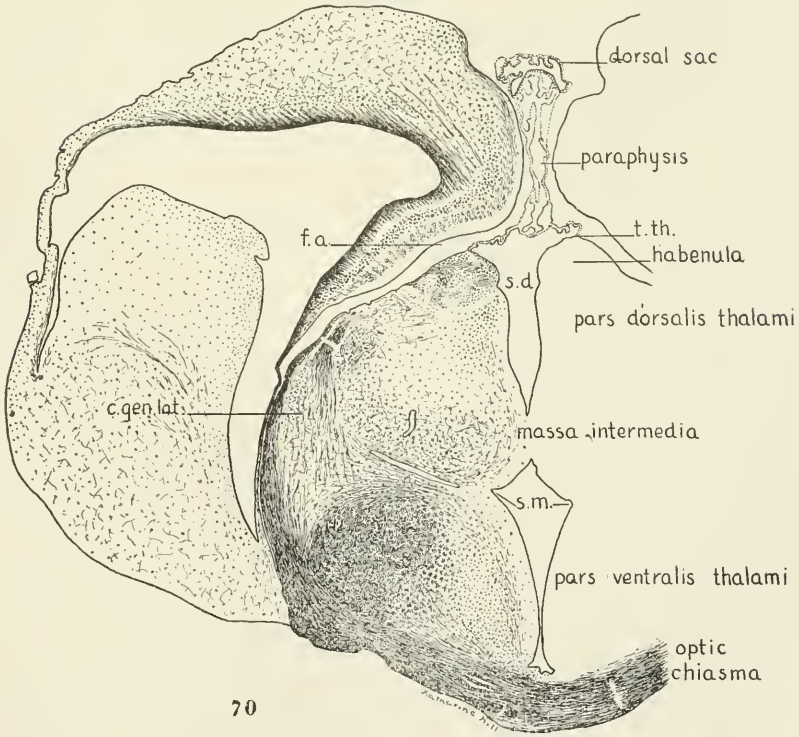
tr.c.hab.
cf.
pars dorsalis thalami
s.m.
n.c.h.
s.v.
t.th.
str.med.
lat.f.b.t.
med.f.b.t.
n.po.
optic chiasma
tr.olf.hab.lat

69


dorsal sac
paraphysis
f.a.
t.th.
habenula
s.d.
pars dorsalis thalami
c.gen.lat.
massa intermedia
s.m.
pars ventralis thalami
optic chiasma

70

EXPLANATION OF FIGURES

71. Diagrammatic cross section through the brain of young Echidna, after Elliot Smith, '03, p. 468, fig. 16.

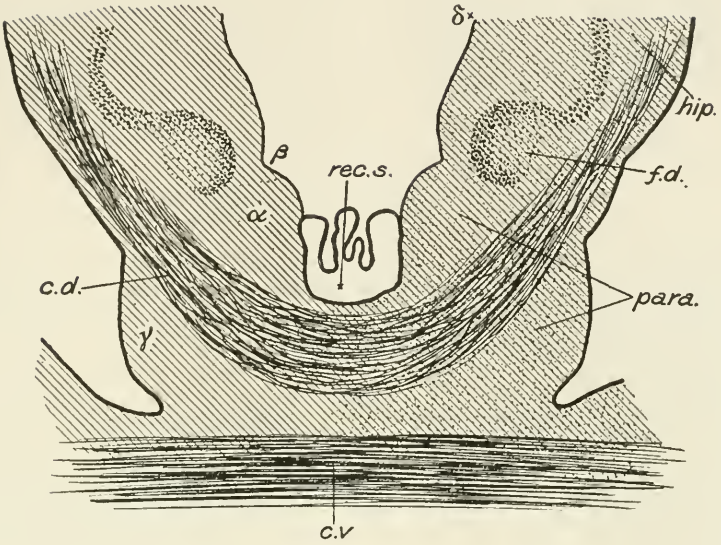
α, dorsal part of the paraterminal body.

β, sulcus limitans hippocampi

γ, ventral part of the paraterminal body.

δ, fissura hippocampi.

72. Diagrammatic median section of the brain of a hypothetical vertebrate ancestor, showing the probable relations of the rostral end of the neural tube before the evagination of the optic vesicles and cerebral hemispheres. The site of the tissue which gives rise to the optic vesicle is shown at *R*, to the olfactory bulb at *O*. (See the text, pp. 468, ff.)


δ
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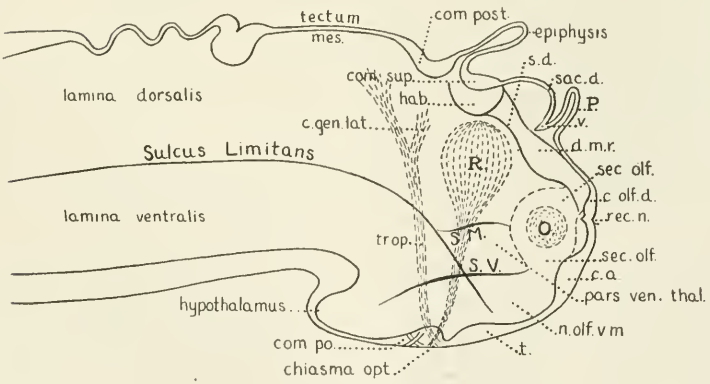
para.
γ
c.v

71


tectum
mes.
com post.
epiphysis
s.d.
com. sup.
sac.d.
lamina dorsalis
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Sulcus Limitans
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R.
sec olf.
c olf.d.
lamina ventralis
rec.n.
trop.
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O.
sec. olf.
S.V.
r.a
pars ven. thal.
hypothalamus
n.olf.v.m
com po.
t.
chiasma opt.

72

EXPLANATION OF FIGURES

73. A diagram similar in plan to that of fig. 72, reconstructed from actual sections of the cyclostome brain, Ichthyomyzon concolor. It is based on the same series of cross sections illustrated by figs. 74 to 81 and the planes of the sections figured are indicated on this figure. (See the text, pp. 470, ff.)

74 to 81. A series of cross sections through the forebrain of a specimen of Ichthyomyzon concolor (Kirtland) 120 mm. long. Drawn from haematoxylin preparations made by Dr. Charles Brookover. × 30. The plane of each of these sections is indicated on fig. 73. The series of sections is numbered, beginning with no. 1 at the lamina terminalis and the serial number of each section figured is given in the following descriptions, the sections being 15 μ thick. Cf. the series of cross-sections of Lampetra figured by Johnston, '02.

74. Section no. 3, immediately behind the lamina terminalis.

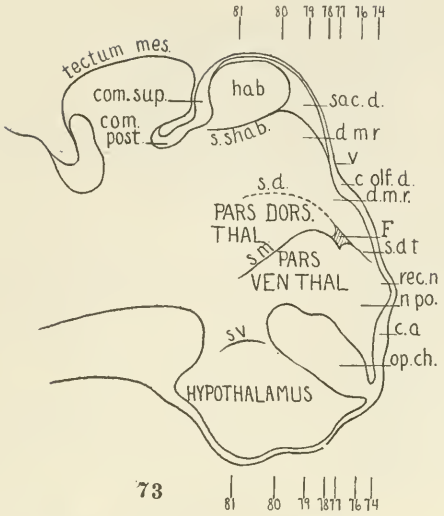
75. Section no. 4, through the recessus preopticus.

76. Section no. 7, through the rostral end of the optic chiasma.

77. Section no. 13, at the rostral border of the interventricular foramen.

78. Section no. 17, immediately caudad of the foramen and passing through the probable site of the embryonic velum transversum.

79. Section no. 23. This section is wholly diencephalic except the lateral hemispheres. The dorso-median ridge here attains its greatest size.



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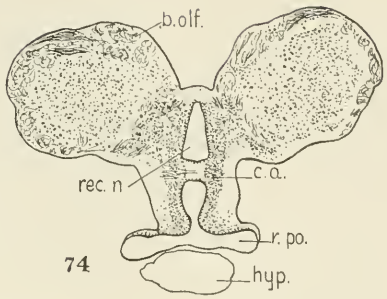

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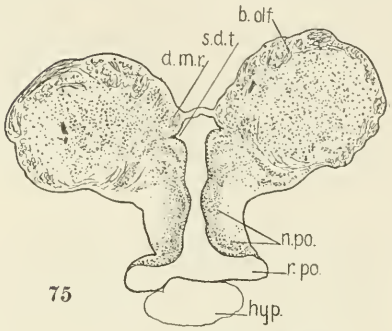

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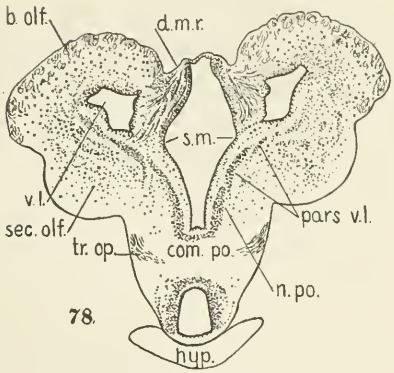
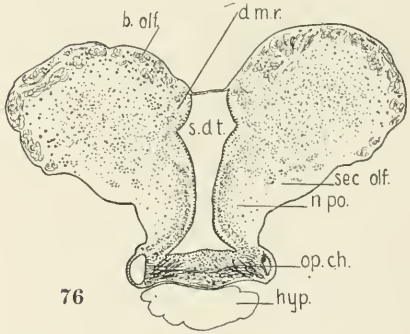


78.



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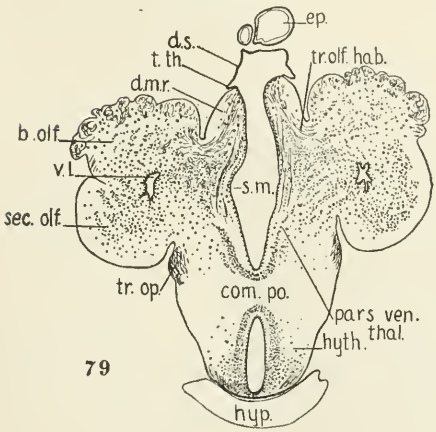

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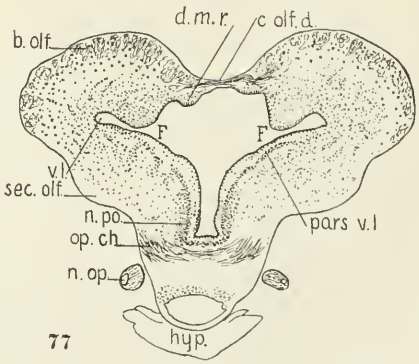


77

EXPLANATION OF FIGURES

80. Section no. 29, through the caudal border of the chiasma ridge. The dorso-median ridge and the pars dorsalis thalami cannot be clearly separated at this level.

81. Section no. 41, through the middle of the habenulae.

82. View of the inner surface of the brain of a human embryo of 6.9 mm. Drawn from the wax model by Ziegler of the embryo Br 3 of the His series and lettered after His, '04, p. 56, fig. 34. The region marked *C.s.* includes not only the corpus striatum but also the preoptic nucleus and other parts of the rhinencephalon.

(1) Margo reuniens (rhinencephalon with rhinencephalon and pallium).

(2) Margo thalamicus (pallium with thalamus, site of the di-telencephalic fissure).

(3) Margo peduncularis (corpus striatum with thalamus, site of the sulcus diencephalicus medius).

(4) Margo hypothalamicus (corpus striatum with hypothalamus).

83. Diagrammatic cross section through the diencephalon of Urodela and Anura. On account of the diencephalic flexure, the section must be taken obliquely to the long axis of the brain in the plane *A-B* of fig. 22, in order to pass transverse to the thalamic axis. The numbers 1, 2, 3 and 4 and the letters *A*, *B* and *C* mark corresponding structures in figs. 83 and 84, the two figures being designed to illustrate the way in which the cerebral hemispheres have been formed by the lateral evagination of the walls of the neural tube; see the text, p. 477.

A, sulcus diencephalicus dorsalis.

B, sulcus diencephalicus medius.

C, sulcus diencephalicus ventralis.

D, roof plate.

V, floor plate.

84. Diagrammatic cross section through the cerebral hemispheres in front of the lamina terminalis of the frog.

A, dorsal angle of hemisphere.

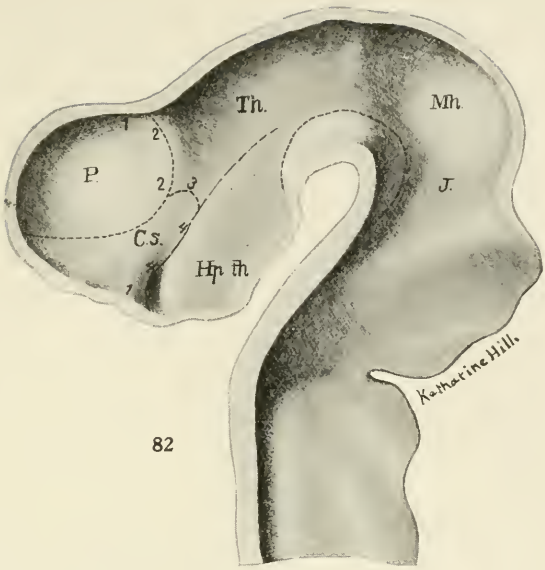
B, zona limitans lateralis and fissura endo-rhinalis.

C, ventral angle of hemisphere.

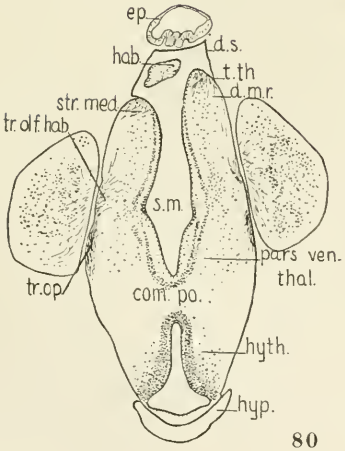
D-V, zona limitans medialis and fissure limitans hippocampi.

L.F.T., lateral forebrain tract.

M.F.T., Medial forebrain tract.


Th.
Mh.
P
J.
Cs.
Hp th
Katharine Hill

82


ep.
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t.th
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str. med.
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s.m.
pars ven. thal.
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com. po.
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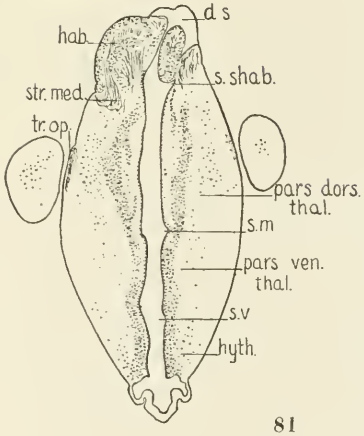
hyp.

80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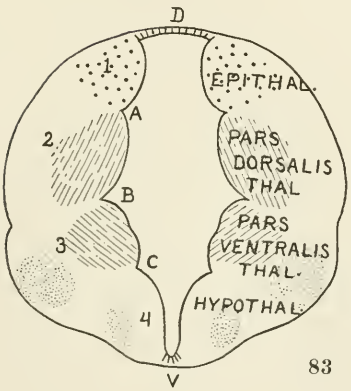
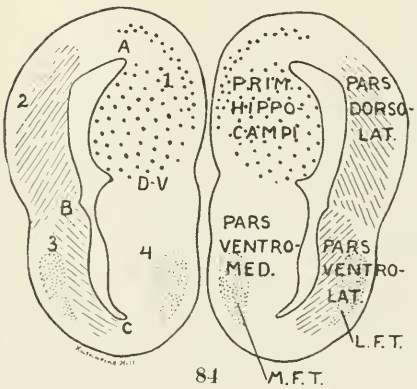
81


D
1
EPITHAL.
A
2
PARS DORSALIS THAL
B
3
PARS VENTRALIS THAL.
C
4
HYPOTHAL.
V

83


A
1
2
D-V
B
3
4
C
PRIM. HIPPO-CAMPI
PARS DORSO-LAT.
PARS VENTRO-MED.
PARS VENTRO-LAT.
L.F.T.
M.F.T.

84

THE RÔLE OF VISION IN THE MENTAL LIFE OF THE MOUSE

KARL T. WAUGH

From the Harvard Psychological Laboratory

TEN FIGURES

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I. PROBLEMS, METHODS AND RESULTS

The purpose of the investigation which is described in this paper was to answer the general question—What does the mouse receive from the outer world through the sense of vision, and of what importance in its life are the visual data so received?

The experimental work was done in the Harvard Psychological Laboratory between January, 1905, and March, 1907. I am

indebted to Professor R. M. Yerkes, under whose immediate supervision the work was carried on, for the suggestion of the problem and for much helpful criticism and advice throughout the course of the work. I wish also to make acknowledgment to Professor Münsterberg and Professor Holt for their advice and suggestions. The anatomical portion of the work was done in the Museum of Comparative Zoölogy at Harvard under the direction of Professor G. H. Parker, to whom I am indebted for aid and advice.

The general method pursued was that of presenting to the mouse a choice between two conditions, one being agreeable to the animal and the other disagreeable. In the majority of cases these were food and a slight electric shock.

PROBLEM 1. DISCRIMINATION OF LIGHT INTENSITY

A. Under indirect illumination

In experiments (a) and (b) under this head, five animals were used.

MOUSE	COLOR	SEX	AGE	DURATION OF EXPERIMENT
No. 1	gray	male	3½ months	Mar. 18–Apr. 17, 1905
No. 2	black	female	3½ months	Mar. 23–Apr. 27, 1905
No. 4	black	male	2 months	Mar. 23–Apr. 27, 1905
D	white[1]	male	3 months	Jan. 21–31, 1906
O	brown	male	3 months	Jan. 17–26, 1906

[1] All the white mice are albinos.

APPARATUS: This consisted of a wooden box measuring 52 cm. x 40 cm. on the inside, and 18 cm. deep. At one end of the box was a small opening fitted with a sliding door, which, when lifted, permitted the mouse to enter from the nest box N (fig. 1). At the other end of the experiment box were arranged two round tin boxes X and Y, each measuring 4½ cm. in diameter, covered with papers which differed in brightness, in color, or in both. The boxes were fitted into small wooden mounts fastened to two boards (22½ cm. x 18½ cm.). On these boards wires were placed, as is shown in fig. 1. These wires were then connected with a Porter inductorium. By closing the key, K, the experimenter could give a mouse whose feet rested upon two adjacent wires a slight shock.

METHOD: Food was placed in one of the boxes and the electric current was switched into the wires on the board which supported

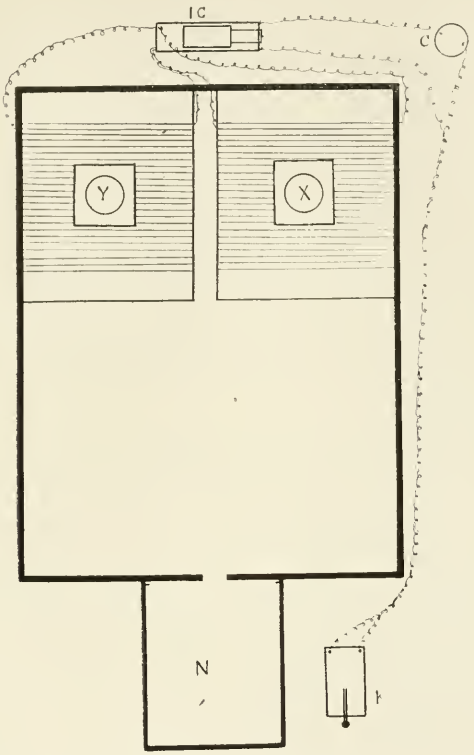

FIG. 1 Apparatus to test visual discrimination in the mouse. *N*, nest box; *X* and *Y*, food boxes; *I.C.* inductorium; *C*, dry cell; *K*, key.

the other box. In this way inducement was given to cause the animal to make use of what discriminative power it might possess for the purpose of avoiding the shock and obtaining the food. This, of course, presupposes the possibility of the formation of an association of shock with one intensity of light and of food with the other. The food used in this experiment was a small quantity of "force," which has little or no odor to enter as a disturbing factor.

An animal was placed in box *N* and the door was lifted. The

mouse would enter the experiment box and make a choice of X or Y, and after receiving a morsel of food or a shock, as the case might be, it would run or be driven back into box N. The boxes X and Y would then be interchanged and the current switched into the wires on the other side. When all was in readiness, a door between N and the main box was raised and the mouse was permitted to seek the food again.

It was considered a choice if the mouse touched the edge of either food-box. If he approached the wrong side first and received a shock and then ran over to the other side to get food, it was recorded as a wrong choice and the animal was forced to return to box N before making the next choice. Twenty trials were made each day with each mouse, and each choice was recorded as right or wrong according as the mouse obtained the food or the shock on first running out.

Results: The following tables give the number of trials and the number of right and wrong choices:

Experiment (a) Black and white.

Black and white papers were pasted on the food-boxes.

MOUSE	FOOD IN	NO. TRIALS	RIGHT CHOICES	WRONG CHOICES	
D.....	white	100	73	27	(see curve, fig. 2)
O.....	white	100	83	17	(see curve, fig. 2)

Experiment (b) Light and dark varieties of a color.

Light and dark violet papers were substituted for the black and white.

MOUSE	FOOD IN	NO. TRIALS	RIGHT CHOICES	WRONG CHOICES	
No. 1.....	Light violet	370	252	118	(see curve, fig. 2)
No. 2.....	Light violet	80	42	38	
No. 4.....	Light violet	230	152	78	(see curve, fig. 2)

Check Experiment: In order to make sure that the animals were using the visual sense in discriminating one paper from

the other, and not the sense of smell, check tests were made. With a mouse that seemed to give good evidence of discrimination, the food was placed in the darker box after having been in the lighter for earlier choices. In the case of mouse no. 1, after a training of 370 trials favoring the lighter violet, and after the boxes had been washed and clean papers pasted on them, the resulting choices were, in ten trials:

Light violet (no food) . . . . . 8 Dark violet (food) 2

In fig. 2 are given the error curves for the discrimination of intensity. The ordinates represent the number of wrong choices in each twenty trials given on a particular day. The succession of days is marked on the axis of abscissas. D and O illustrate experiment (a); no. 1 and no. 4 illustrate experiment (b).

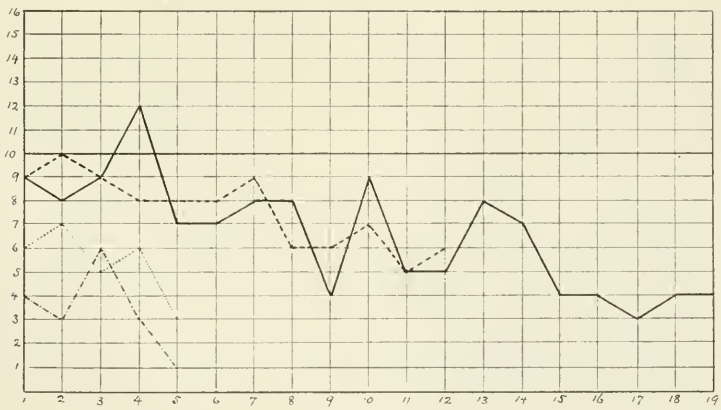

FIG. 2 Error curves for discrimination of light intensity. Ordinates represent number of wrong choices in twenty trials; abscissas represent days

————————, Curve for mouse No. 1.
– – – – – – – – – – – – , Curve for mouse No. 4.
- - - - - - - - - - - - - - - -, Curve for mouse D.
—·—·—·—·—·—·—·—, Curve for mouse O.

Experiment (c) Influence of background.

METHOD: In this experiment two animals, O and D, were used. They were trained for white by one hundred trials, in which the back and sides of the experiment box were covered with cards

(18 x 18 cm.). These were interchanged when the food boxes were changed so that the black food box always had a black background and the white box appeared against a white background. The choices during this training were not recorded.

Upon the completion of the training, changes were made in order to obtain an answer to the questions: does the mouse choose by discriminating between white and black boxes, or is it influenced also by the illumination of the whole field? Is food associated with object or with background? For this purpose white cards were placed behind the black box and black cards behind the white. Other conditions were later introduced for testing the nature of the association formed.

RESULTS:

White box against black background and vice versa

MOUSE	NO. TRIALS	RIGHT	WRONG	REMARKS
O	20	15	5	The last ten, 100 per cent
D	20	13	7	right, *i.e.*, white.

Uncovered tin boxes used. Backgrounds only changed

MOUSE	NO. TRIALS	RIGHT	WRONG	REMARKS
O	10	7	3	Great hesitation
D	10	10	0	

Mouse D was now tried without backgrounds but with the black and white papers on the food boxes. The result of twenty such choices was:

White box.....14 Black box.....6

The next experiment was with plain tin boxes without backgrounds, but with strips of paper (4 cm. x 18 cm.) laid crosswise on the floor of the experiment box directly in front of the boards which carried the electric wires. Black paper was placed on the one side and white on the other.

Under these conditions mouse O in ten trials crossed over the white paper to reach the tin box nine times, the black paper, once.

In the next experiment, light gray paper was substituted for the white with the following results.

MOUSE	NO. TRIALS	LIGHT GRAY	BLACK
C	26	12	14
D	10	6	4

The strips were now taken up and the tin boxes were covered respectively with light gray and dark gray papers. No backgrounds were used.

The experiment resulted as follows: By mouse D, in 40 trials, the light gray was chosen 19 times, the dark gray 21 times.

Experiment (d). Selection of yarns.

Preference of mice for light or dark yarns obviously depends upon their power of discrimination.

METHOD: Neither food nor electric shock was used in this experiment. Advantage was taken of the opportunity afforded by the instinct of a mother mouse to make a warm nest for her litter. A black mouse, X, about six months old, with five little ones, was taken as subject. When the young mice were a little over a week old, pieces of yarn were hung in the cage and some of the cotton was removed from the nest. The order in which the mouse took the different yarns to replenish the nest was recorded.

The yarns used were white, black, and two shades of gray. These were hung in the cage in a row, 4 cm. apart and at a distance of 15 cm. from the entrance to the nest. The lower ends rested on the floor of the cage. After a set had been pulled down by the mouse and taken into the nest, four other pieces were hung up in a different order. After three or four sets had thus been taken they were removed from the nest and the experiment was repeated.

In an entire series all the possible arrangements of the four shades were made. There being six possible permutations of the four yarns and four places, there were 96 selections in the series, each yarn appearing in each place six times. The order in choosing the four positions was recorded also, in order to show what influence the habit of first seeking a certain locality might have.

RESULTS: The table gives the number of times each shade was selected first, second, third, and fourth or last.

YARN	FIRST CHOICE	SECOND CHOICE	THIRD CHOICE	FOURTH CHOICE
Black	8	7	7	2
Dark gray	6	6	7	5
Light gray	4	2	6	12
White	6	9	4	5

As seen from the table, the order of preference is (1) black, (2) white, (3) dark gray, (4) light gray.

The nest was next turned through 180 degrees and the yarns were hung in the back of the cage, opposite where they had been before, and another complete series of records of choices was obtained in the same manner. The results of this second series are summarized in the following table:

YARN	FIRST CHOICE	SECOND CHOICE	THIRD CHOICE	FOURTH CHOICE
Black	7	6	8	3
Dark gray	5	8	7	4
Light gray	3	4	3	14
White	9	6	6	3

The order of preference as shown from this table is (1) white, (2) black, (3) dark gray, (4) light gray.

The leaving of light gray till last in both series was so interesting that it was thought well to make a check test to learn whether the taste or odor of that particular dye was determining the animal's choice rather than the shade. A gray yarn was made by twisting together a strand from the black and one from the white, the two preferred yarns, and a set of 24 choices of the three yarns, black, white and gray, was obtained.

The results were as follows:

YARN	FIRST CHOICE	SECOND CHOICE	LAST
White	5	0	1
Black	1	3	2
Gray (black and white)	0	3	3

Conclusions: (From the experiments under problem, 1, A.)

That the mouse discriminates between light and dark objects under indirect illumination is evident from experiments (a) and (b).

Experiment (c) shows that both object and background are influential in determining the reaction.

The albino mouse was influenced by the environment more than the brown mouse. This is shown in the case of the white mouse D making 100 per cent right choices, *i.e.*, choices of white background when the food boxes were uncovered. This result is quite in harmony with the biological theory of protective coloration.

B. Under direct illumination

Apparatus: In fig. 3 is shown a view of the apparatus used in these experiments. It consists of two parts, an experiment box (32 cm. x 52 cm.) and a light box (32 cm. x 98 cm.) Between these two parts is a slide carrying ray filters. *A* is a nest box ($29\frac{1}{2}$ cm. x 18 cm. inside) from which the animal can enter the compartment *B* through a door *I*. From *B* (20 cm. x 17 cm.) it cannot pass back into *A* directly, but must enter one of the smaller compartments in front, which open into alleys on each side. From one of these alleys the animal reaches the nest box by a gate *O*. The two small compartments, (each $14\frac{1}{2}$ cm. x 8 cm.) which may be entered from *B*, are illuminated by the light from electric lamps in the light box, which enters the compartments at *G* and *R* through two apertures each $6\frac{1}{2}$ cm. square. These apertures, in the experiments now to be described, were covered with ground glass. In the light box the lamps can be moved back and forth to give the required differences in intensity, their distance from the ground glass being measured on a scale *S*. The light box is divided lengthwise by a partition which insures the illumination of each aperture by the appropriate lamp only. The slide carries three rectangular cells (15x16 x 6 cm.) separated from one another by pieces of felt. These, filled with colored media, can be used as ray filters for tests of color discrimination. During the present experiments they remained empty.

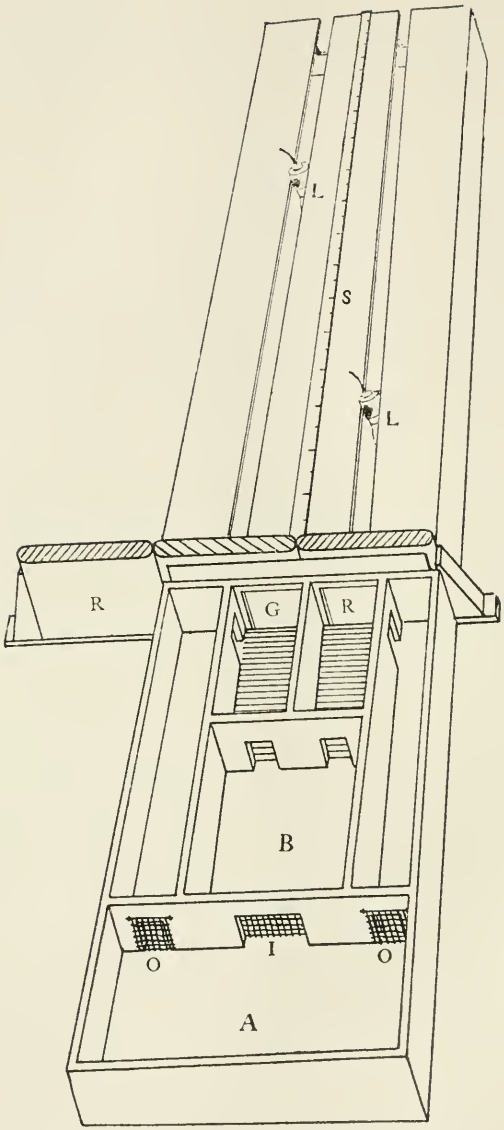

FIG. 3 Visual discrimination apparatus. *A*, nest box; *B*, entrance chamber; *R*, *R*, red ray-filter; *G*, green ray-filter; *L*, *L*, incandescent lamps in light box; *S*, millimeter scale on light box; *I*, door between *A* and *B*; *O*, *O*, doors between alleys and *A*. (Yerkes. The Dancing Mouse, p. 153. 1907. The Macmillan Co., N. Y.)

METHOD: The method is very similar to that previously described. Two animals were used:

MOUSE	COLOR	SEX	AGE
U	black	male	About 4 months
V	white	male	5 months

These animals were trained to enter the brighter of the two compartments. Food was kept in both alleys near *O*, but the exit into the alley on the side of the weaker light was closed by a piece of glass which could be slipped into the partition just above the exit and pushed down. A shock was given whenever the mouse entered the darker compartment. From the brighter compartment the animal was allowed to pass unmolested into the alley, obtain food and enter the nest box through the passage *O*. After receiving a shock the animal was apt to run quickly out of the darker compartment and into the other one.

The wire door at *I* permitted passage only into *B*, and those at *O*, *O*, only into *A*. The mice soon learned that the food was to be obtained only by passing from *B* through one of the small compartments into the alley.

Twenty experiments were made with a mouse each day. Sometimes it was found necessary to urge the animal to make a choice by gradually narrowing the space in compartment *B* with a thin board, placed vertically across the compartment.

The experiment was begun with one light at a distance of 34 cm. from the ground glass, and the other at 54 cm. After each choice, or sometimes two or three choices, the light that had been at 34 cm. was moved to 54 cm. and the other moved up to 34 cm. thus making the other compartment the brighter. At the same time the piece of glass which had blocked the exit was removed and pushed into the other slip, blocking the exit on the opposite side.

RESULTS: ("Right" indicates choice of the brighter of the two lights).

Lights at 34 cm. and 54 cm.

MOUSE		CHOICES 1ST DAY	2ND	3RD	4TH	5TH	TOTAL
U	Right	15	17	14	17	18	81
	Wrong	5	3	3	3	2	19
V	Right	14	14	13	17	18	74
	Wrong	6	6	7	3	4	26

Lights at 34 cm. and 40 cm.

MOUSE		CHOICES 1ST DAY	2ND	3RD	4TH	5TH	TOTAL
U	Right	15	12	13	13	14	57
	Wrong	5	8	7	7	6	33
V	Right	11	13	13	12	13	62
	Wrong	9	7	7	8	7	38

CONCLUSIONS: The mouse discriminates between differences in the brightness of white light; the less the objective difference, the greater the difficulty in discrimination.

The discrimination of the albino mouse is slightly inferior to that of the mouse with black eyes.

PROBLEM 2. COLOR DISCRIMINATION

A. Under indirect illumination

Experiment (a). Discrimination of colored objects.

APPARATUS AND METHOD: The apparatus used in this experiment was the same as that already described under problem 1, A, (a) and (b) (see p. 550). The colors chosen were an orange-red and a blue (Bradley papers). These were judged to be of equal intensity by several members of the laboratory. The papers were pasted on tin boxes and the experiment was conducted as in the intensity-discrimination experiment. The following eleven mice were used:

MOUSE	COLOR[1]	SEX	AGE[2]	DATE OF EXPERIMENT
No. 1	gray	male	2 months	Jan. 27–Feb. 20, 1905
No. 3	gray	male	7 weeks	Feb. 17–25, 1905
W F	white	female	7 weeks	Feb. 2–25, 1905
No. 5	gray	male	9 weeks	Nov. 20–Dec. 2, 1905
B M	brown	male	2 months	Oct. 10–Nov. 8, 1905
B S	brown	male	1 month	Oct. 10–Nov. 8, 1905
A	white	male	adult	Dec. 2–4, 1905
B	white	male	adult	Dec. 2–4, 1905
C	white	male	1 month	Nov. 9–Dec. 10, 1905
D	white	male	5 weeks	Nov. 9–Dec. 10, 1905
E	white	male	7 weeks	Nov. 29–Dec. 10, 1905

[1] All the white mice are albinos.

[2] The ages are approximate. An adult mouse is over 2 months old. Age given is age at the time experiment was begun.

Marked individual differences in the animals made it possible to get a much greater number of choices with some than with others. The ease with which a motor habit may be formed makes all the difference between a good subject and a poor one. Mouse no. 1 early formed the habit of running directly to one of the tin boxes, taking a piece of food (if he happened to select the box which contained food) and running back with the morsel to box *N*. He would be eating the last piece of food while I was changing the boxes and switching the current to the other board. Thus he saved me much time. He always made his full assignment of twenty choices per day. Some of the other mice would follow along the edge of the experiment box, pause a long time in the corners and wash their faces, or they would take the food in such a way as to give no satisfactory evidence of choice. Throughout these red-blue discrimination tests the food was kept in the red box. At frequent intervals the papers were removed and fresh ones pasted on, in order to obviate the influence of odor. The experiment box was so adjusted with reference to the windows of the room, that the two halves were equally illuminated.

Results: The following table gives a summary of the results of the red-blue discrimination tests:

MOUSE	NO. TRIALS	RIGHT (RED)	WRONG (BLUE)	PER CENT RIGHT
No. 1	480	320	160	67
No. 3	55	38	17	70
W F	185	104	81	56
No. 5	91	49	42	54
B M	210	160	50	76
B S	28	15	13	54
A	12	9	3	75
B	10	6	4	60
C	55	29	26	53
D	44	22	22	50
E	120	68	52	57

The figures in this table represent the total number of right and of wrong choices during the training. The improvement in discrimination and the stage of success finally attained are shown in the curves of fig. 4. The results for one mouse are presented as typical. On the axes of ordinates are represented the

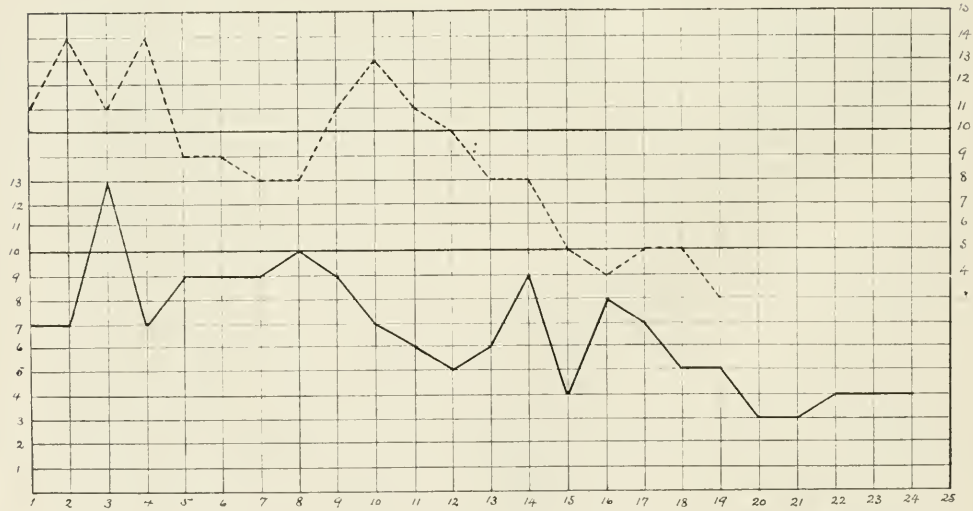

FIG. 4 Curves of learning. Number of errors made daily is represented on the axis of ordinates. Number of days is represented on the axis of abscissas. Lower curve is the error curve for one mouse in red-blue discrimination. Upper curve is the curve of dissociation and association for the same mouse.

number of errors made daily in twenty choices. Successive days on which sets of choices were made are represented on the axis of abscissas. The horizontal line at 10 may be regarded as the line of no discrimination.

Check tests: To make sure that vision was being used by the animals in discriminating, rather than smell, food was placed in the blue-covered box and the red box was emptied. With mouse no. 1 after the training for red of 400 trials, the result was, that, in the first hundred choices, the mouse selected the empty red box first 59 times in preference to the blue which contained the food.

Keeping the food in the blue, the experiment was continued in order to discover how rapidly an association of one color with food, formed through training, could be changed to an association involving a preference for the previously avoided color. The dotted curve of fig. 4 represents the result of 380 trials. It is an error curve in which choices of red are considered "wrong."

With another mouse, B M, a series of experiments was made, in which the check test consisted in changing the food into the blue box for from three to five choices, after each ten choices with the food in the red. This was done after 190 choices in training for red had already been made. The results are given in the following table. The first column gives the number of the test; the second gives the number of right choices out of ten with the food in red; the last two columns give the results with the food transferred to the blue.

NO.	FOOD IN RED	FOOD IN BLUE	
	Right Choices	No. trials	Red chosen
1	8	5	5
2	9	5	3
3	8	5	5
4	10	5	4
5	7	5	3
6	8	5	5
7	4	5	3
8	9	3	0
Total	63	38	28

Experiment (b) Selection of colored yarns.

METHOD: The apparatus as well as the method here used was the same as that used in the experiment on the selection of gray yarns (see p. 555). Three animals, all of them mothers of litters, were subjects in the experiment.

MOUSE	COLOR	SEX	AGE	DATE OF EXPERIMENT
K	white	female	3 months	Mch.–Apr., 1906
X	black	female	6 months	Jan., 1907
Y	white	female	cir. 1 year	Jan., 1907

Four colors, blue, red, yellow and green, of as full saturation as possible, were selected from among the yarns used in the laboratory for testing color blindness. These were hung in a row in the back part of the mouse's cage, all at the same distance from the opening into the nest. After the four pieces had been pulled down and carried into the nest one after another, another set was arranged in a different order, as in the previous experiment with gray yarns.

RESULTS: These are presented in tables, each table representing a series of 96 selections of yarns.

Mouse K

COLOR	NO. OF TIMES SELECTED				ORDER OF PREFERENCE
	1st	2nd	3rd	4th	
Blue	6	3	10	5	3
Red	10	5	3	6	1
Yellow	6	11	2	5	2
Green	2	5	9	8	4

Mouse X

COLOR	NO. OF TIMES SELECTED				ORDER OF PREFERENCE
	1st	2nd	3rd	4th	
Blue	7	5	4	8	3
Red	6	11	3	4	2
Yellow	11	3	7	3	1
Green	0	5	10	9	4

The order of preference is obtained by comparing the numbers which represent the *preference-value* of each color. Such a number may be obtained by adding together the number of times a color is chosen first $\times 4$, the times it is chosen second $\times 3$, the times chosen third $\times 2$ and the number of times chosen last. Thus for K, the preference value of blue is: $6 \times 4 + 3 \times 3 + 10 \times 2 + 5 = 58$. In the same way the preference value of red is found to be 67, of yellow 66, and of green 49. For X the values are: blue 59, red 67, yellow 70, and green 44.

This preference-value is useful in showing the constancy throughout the series. Thus the whole set of results for X was divided into halves and the preference values for each half were as follows:

	FIRST HALF	SECOND HALF	WHOLE
Blue	26	33	59
Red	34	33	67
Yellow	35	35	70
Green	25	19	44

Conclusions: The mouse can learn to associate food or electric shock with red or blue objects. The connections thus formed may be disassociated and an association formed with another color.

In albino mice, color discrimination is poor.

Red and yellow are preferred to blue and green, and of the latter two, blue is preferred to green.

Whether the discrimination involved is true color discrimination, after the fashion of that in human beings, can not be discovered, but we may call it color discrimination so long as it answers the practical purposes of the mouse in distinguishing between such objects as it is likely to meet with in its habitat.

If it be claimed that colors of the red end of the spectrum are preferred by the black mice because they seem to them the darker, we would suggest a correlation with the results obtained in the matter of the preference for gray yarns (see pp. 556). The same mouse, X, appeared in the two experiments. White and black were both preferred to either of the intermediate grays.

B. Under direct illumination

Apparatus: The same as that in problem 1, B, fig. 3. The colors used were red, blue and green, obtained by means of the following ray filters and glasses:

For green—a saturated solution of nickel nitrate.

For blue—solution of copper ammonia sulphate.

For red—pieces of ruby glass placed in the cell.

The three cells in the slide between the light box and the experiment box were filled with the solutions, the two on the outside containing the same solution. By moving the slide a little to one side, the relative positions of the colors were changed, the one previously on the right now appearing on the left. The cell containing the ruby glass was filled with water in order that by the effect of the refraction it might appear to be the same distance away as the other colors.

In all, fourteen animals were made use of in this experiment:

MOUSE	COLOR	SEX	AGE	DATE OF EXPERIMENT
Q	white	male	adult	Apr. 23–27, 1906
J	white	male	adult	Apr. 17–May 17, 1906
N	black	male	adult	Apr. 28–May 7, 1906
R	white	male	adult	Apr. 12–May 25, 1906
G	brown	female	adult	Apr. 5–May 19, 1906
C	white	male	5 months	Mar. 26–May 24, 1906
M	brown	male	? (wild)	Apr. 6–9, 1906
D	white	male	7 months	Mar. 26–May 24, 1906
F	brown	male	adult	Apr. 9–May 19, 1906
T	gray	male	4 months	Nov. 9–Dec. 17, 1906
U	black	male	4 months	Nov. 25–Jan. 14, 1907
Xa	gray and white[1]	male	1 month	Jan. 9–21, 1907
V	white	male	6 months	Nov. 16–Dec. 10, 1906
Z	white	male	6 months	Nov. 14–Dec. 10, 1906

[1] The gray and white mouse had pigmented eyes.

Method: The first two colors used were green and red. One of the electric lamps was moved until the intensities of the two colors appeared equal. Judgment was given on this point by five members of the laboratory, after each had been in the dark-room for five minutes.

A preliminary test of 50 trials was made with each animal to see if any natural preference for either of the two colored lights existed. The mice were found negative in this respect. Under the circumstances, it is evident that the problem resolves itself into the question whether the mouse can be trained to prefer one quality of light to another. This is to be done by the association of pleasure or pain with whatever distinguishing characteristics the lights may present to the eye of the animal. Just what the distinguishing characteristics are—what factors the mouse uses in discriminating—may be suggested by the use of check tests, in which the relative brightness of the lights is varied. In this respect the apparatus of this experiment is more satisfactory than that of the first series under this problem. Yet it leaves something to be desired, for if the animal's choices are determined wholly by intensity, this fact would become apparent, but if they are determined by more than one factor (*e.g.*, quality and intensity) as seems probable, then we can hope for results only on the supposition that a point of non-discrimination may be found, or better, a point of least discrimination, where a certain intensity tends to counteract the quality which would act in part as a determining factor.

RESULTS:

Red-green discrimination

MOUSE	TRAINED FOR	NO. CHOICES	RIGHT	WRONG
Q	green	100	57	43
J	red	100	60	40
N	red	100	58	42

Green-blue discrimination

MOUSE	TRAINED FOR	NO. CHOICES	RIGHT	WRONG
R	green	100	42	58
G	green	100	50	50
C	green	100	49	51
M	green	60	29	31
D	green	100	50	50
F	green	80	41	39

The next problem undertaken was that of ascertaining whether the mouse discriminates between white and red lights when they are of about the same degree of brightness.

For this experiment four incandescent lamps were used as sources of light. Two, measuring 4 c.p. each, were used back of ruby glass to yield the red light, and two measuring 13 c.p. each back of ground glass to yield the white light stimulus. The positions of these lamps in the light box were changed as indicated in the following tables of results.

Lamp of 13 c.p. 34 cm. from red glass; lamp of 4 c.p. 80 cm. from white glass (This yielded a red and a white stimulus which seemed of equal intensity to the human observer)

MOUSE	COLOR	NO. OF TIMES CHOSEN										TOTAL
T.....	White	6	4	8	6	7	7	6	8	6	7	65
T.....	Red	4	6	2	4	3	3	4	2	4	3	35

Lamp of 13 c.p. 14 cm. from red glass; lamp of 4 c.p. 90 cm. from white glass

MOUSE	COLOR	NO. OF TIMES CHOSEN												TOTAL
U.....	White	8	9	8	8	7	7	8	8	10	8	9	10	100
U.....	Red	2	1	2	2	3	3	2	2	0	2	1	0	20
T.....	White	8	6	7	7	–	–	–	–	–	–	–	–	28
T.....	Red	2	4	3	3	–	–	–	–	–	–	–	–	12

Lamp of 13 c.p. 54 cm. from the red glass; lamp of 4 c.p. 70 cm. from white glass

MOUSE	COLOR	CHOICES		TOTAL	MOUSE	COLOR	HOICES		TOTAL
U.....	White	7	10	17	T.....	White	8	9	17
U.....	Red	3	0	3	T.....	Red	2	1	3

Lamp of 13 c.p. 14 cm. from red glass (one of the two thicknesses of ruby glass used previously removed); lamp of 4 c.p. 90 cm. from white glass

MOUSE	COLOR	CHOICES		TOTAL
U.....	White	8	8	16
U.....	Red	2	2	4

Lamp of 13 c.p. 14 cm. from red glass (single thickness); no light through white glass

MOUSE	COLOR	CHOICES			TOTAL
U	White	6	3	6	15
U	Red	4	7	4	15

Lamp of 16 c.p. 14 cm. from red glass; lamp of 1.69 c.p. 90 cm. from white glass.

MOUSE	COLOR	CHOICES		TOTAL
U	White	8	9	17
U	Red	2	1	3

Lamp of 16 c.p. 14 cm. from red glass; Lamp of 1.69 c.p. 90 cm. from white glass. (With glasses at exits, so placed as not to reflect light)

MOUSE	COLOR	CHOICES									TOTAL
U	White	4	9	6	5	6	6	6	7	–	–	49
U	Red	6	1	4	5	4	4	4	3	–	–	31
Xa	White	7	6	5	8	5	5	5	4	5	6	56
Xa	Red	3	4	5	2	5	5	5	6	5	4	44

Lamp of 16 c.p. 34 cm. from red glass; lamp of 1.69 c.p. 60 c.m. from white glass

MOUSE	COLOR	CHOICES			TOTAL
Xa	White	5	4	6	15
Xa	Red	5	6	4	15

Lamp of 13 c.p. 34 cm. from red glass; lamp of 4 c.p. 80 cm. from white glass (Brightnesses equal for human eye)

MOUSE	COLOR	CHOICES										TOTAL
V	White	5	5	6	6	6	5	8	4	5	7	57
V	Red	5	5	4	4	4	5	2	6	5	3	43
Z	White	6	8	4	4	7	3	5	5	5	6	53
Z	Red	4	2	6	6	3	7	5	5	5	4	47

Conclusions: Our results do not indicate any ability to discriminate between red and green or between blue and green. The black-eyed mice discriminate between red and white light when they are of equal brightness to the human eye. When the red is made darker the discrimination is slightly improved. When the red is made brighter the discrimination is not so good. The power to discriminate seems to fall away as the brightness of the red relative to the white increases for the human observer.

PROBLEM 3. PERCEPTION OF FORM

Apparatus and Method: In these experiments the apparatus used was the same as that already described in connection with the problem of the discrimination of direct light stimuli. (see p. 557)

Pieces of black cards were substituted for the liquids in the cells, and in these cards were cut holes of the forms desired for the test. The forms selected were, a circle, 4 cm. in diameter, and an X-shaped figure which was inscribable in a square of 4 cm. diameter (fig. 5). I made these two figures of equal area in

Fig. 5 Cards for study of perception of form.

order that difference in the amount of light passing through them might not serve as a condition of discrimination.

Two incandescent lamps were used as sources of light. Each of these was of 13 candle-power, as tested by the Lummer-Brodhun photometer. The lamps were placed in the light box at a distance of 50 cm. from the apertures. Additional pieces of ground glass were placed against the black cards inside the cells, in order

to make the illumination of the apertures more homogeneous, as well as to reduce the light.

The electric shock (as punishment for a wrong choice) was given in the compartment with the X-aperture, the side exit on that side being closed with a piece of glass. When the animal entered the compartment marked by the circle, it was allowed to pass into the alley and to obtain food.

The circle and X were interchanged at intervals, usually after three or four choices had been made. This was accomplished by sliding the carriage a few inches to the right or left so that another card with an X-aperture would come into the field, taking the circle's place, the circle having moved into the position of the former X. Ten choices were obtained each day. Four mice were used in this experiment.

MOUSE	COLOR	SEX	AGE
Ya	white	female	3 months
Yi	white	female	3 months
X	black	female	5 months
Xd	black	female	6 weeks

RESULTS: In the following table, R stands for right choices, *i.e.*, circle; W for wrong, *i.e.*, X-aperture.

DAY	Ya		Yi		X		Xd	
	R	W	R	W	R	W	R	W
1st	4	6	4	6	3	7	8	2
2nd	7	3	7	3	6	4	4	6
3rd	8	2	4	6	6	4	8	2
4th	8	2	10	0	6	4	7	3
5th	8	2	6	4	5	5	6	4
6th	6	4	6	4	–	–	6	4
7th	8	2	8	2	–	–	–	–
8th	7	3	6	4	–	–	–	–
9th	7	3	7	3	–	–	–	–
10th	6	4	–	–	–	–	–	–
11th	7	3	–	–	–	–	–	–
12th	7	3	–	–	–	–	–	–
13th	7	3	–	–	–	–	–	–
Totals	90	40	58	32	26	24	39	21

Conclusions: As may be seen from the table, perception of form does not seem to be very well developed in the mouse. Only in the case of Ya, may we feel justified in concluding that discrimination was present, and even here errors were not eliminated up to the 130th choice. That the mouse is able to form an association of object with food or shock is shown by the experiments in intensity discrimination. Evidently the mouse tends to depend upon the size of the illuminated area or the intensity of the light.

When a strange mouse is introduced into the cage of another mouse there seems to be no recognition of the nature of the intruder by vision. Not until the home mouse can touch and smell of the stranger, does there seem to be any knowledge of whether he is an enemy or the mate of the home mouse. One might suppose that differences in the form of the animal would be noticed at some little distance. It is by form that human beings known one another, different expressions of the face being in the last analysis minor differences in form.

Problem 4. Perception of Distance of Object from Animal

Apparatus: A wooden disk 10 cm. in diameter, supported in the center by a column $2\frac{1}{2}$ cm. in diameter, which passes through

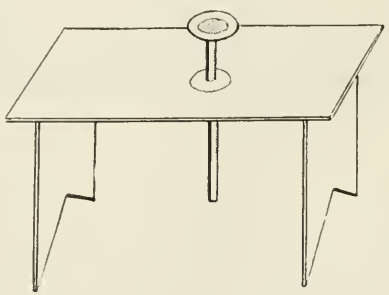

Fig. 6 Table for study of perception of distance.

a round hole in the top of a bench. The height of the disk above the bench can be adjusted by sliding the column up or down.

METHOD: A mouse was placed upon the disk, raised to a certain height above the bench and allowed to jump down. The time which elapsed from the instant the mouse was placed on the disk till he jumped off was recorded with a stop-watch. The time was taken as a measure of the animal's ability to perceive distance. The mice were active and restless and continually peered down over the edge of the board as though measuring the distance of the leap.

Nine animals were used in this experiment:

MOUSE	COLOR	SEX	AGE	DATES OF EXPERIMENT
E	white	male	7 weeks	Nov. 22–Dec. 14, 1905
BM	brown	male	9 weeks	Nov. 22–Dec. 14, 1905
D	white	male	7 weeks	Nov. 22–Dec. 14, 1905
U	black	male	cir. 3 months	Jan., 1907
Xb	gray	female	6 weeks	Jan., 1907
Wa	white	male	3 months	Jan., 1907
Yc	white	female	3½ months	Jan., 1907
Xd	black	male	6 weeks	Jan., 1907
T	gray	male	cir. 6 months	Jan., 1907

With each mouse five trials were made daily, at each of several different heights of the disk, and an average was taken of the time records for these five. Usually if an animal was not off the disk within two minutes it was recorded as unwilling to venture the leap.

RESULTS:

With plain bench-top below disk

HEIGHT OF DISK	18 CM.	15 CM.	12 CM.	10 CM.	8 CM.	6 CM.	4 CM.
	sec.	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*
E's time	35	31	19	7.5	6.4	5.6	2
BM's time	—	45	7	4.5	3.5	3.4	—

Large red and blue sheets of paper, when placed over the top of the bench, gave similar results.

When sawdust and scraps of paper were scattered on the bench, the time was slightly lessened. This, however, may be due to the

greater ease of jumping off, which had been acquired through practice.

The disk with its supporting column was inverted and inserted in the bench-top from below. This was done because it was feared that the animal in peering over the edge of the disk might be influenced by seeing the column. A large plate of glass was fixed under the disk in its new position.

With disk inverted and glass below it

MOUSE	10 CM.	8 CM.	6 CM.	4 CM.
	sec.	*sec.*	*sec.*	*sec.*
E	41	18	—	—
D	120	120	120	7.6

At 4 cm. the vibrissae touched the glass. D's average, even at this height, was unusually long.

When the glass was lowered to a distance of 10 cm. above the floor the results were similar to those just presented.

With a board, instead of the glass, placed below the disk

MOUSE	6 CM.	5 CM.	4 CM.	3 CM.	2 CM.
	sec.	*sec.*	*sec.*	*sec.*	*sec.*
U	120	32	22	—	–
U (2d series)	—	75	31	17	–
Xd	120	120	45	36	2
Xb	120	120	120	5	
Xc	120	69	10	–	–
Xc (2d series)	–	60	33	–	–
T	120	59	32		–

The effect of a white and black bench surface upon the animal's reactions was next tested.

METHOD: With the disk in position above the bench, white and black papers were spread over the bench in such a way that one half of the disk was above white, the other half above black paper. A large piece of plate glass having a round hole in the center for

the column to pass through, was placed over the papers in order that the surfaces might not be soiled by the animals, as well as to keep the papers flat and in position. A circular disk of polished steel, 7 cm. in diameter, was fitted closely around the column over the glass. This was intended to prevent the animals from jumping down too close to the column. A small wooden disk wrapped with electric wires was fastened to the top of the disk to bring about a quicker reaction by making too long a stay on the disk slightly unpleasant for the mouse. The wires from this small disk passed through the larger disk and were sunk into the sides of the column, thence they passed downward through the bench top to the floor where they were connected with an induction coil and dry cell.

The time records are of little importance here, for the purpose of the experiment was merely to observe the choice of the black or white surface as a landing place. It was assumed that the animal would jump down on the surface which appeared to it the nearer.

RESULTS:

Height of disk 4 cm. above bench

MOUSE	TRIAL	PLACE	TIME	STIMULUS
			sec.	
Wa	1	white	10	shock
Wa	2	white	3	no shock
Wa	3	white	5	no shock
Wa	4	joining	10	no shock
Wa	5	white	3	no shock
Wa	6	white	3	no shock

Disk turned 180 degrees

MOUSE	TRIAL	PLACE	TIME	STIMULUS
			sec.	
Wa	7	white	5	no shock
Wa	8	white	67	shock
Wa	9	white	320	shock

The next day another series was tried with the disk in its original position and the whole apparatus turned around 180 degrees.

Disk 5 cm. above the bench

MOUSE	TRIAL	PLACE	TIME	STIMULUS
			sec.	
Wa	1	white	20	no shock
Wa	2	white	5	no shock
Wa	3	white	300	shock

Disk turned 180 degrees

MOUSE	TRIAL	PLACE	TIME	STIMULUS
			sec.	
Wa	4	white	120	shock
Wa	5	white	186	shock
Wa	6	white	1200	shock
Ye	1	white	6	no shock
Ye	2	black	250	shock
Ye	3	joining	15	shock
Ye	4	black	18	shock
Ye	5	joining	17	shock

Table turned 180 degrees

MOUSE	TRIAL	PLACE	TIME	STIMULUS
			sec.	
Ye	6	white	16	shock
Ye	7	joining	3	shock
Ye	8	white	5	shock
Ye	9	hitew	14	shock
Ye	10	white	3	shock

Conclusions: While this is not an entirely satisfactory method of studying the distance perception of the mouse, it makes possible some inferences that are of value. The objections which may be made to the method are that the activity of the animal on the disk, particularly the going round and round while hanging over

the edge, is not a search for the nearest point of the surface below, but merely the activity resulting from a kind of agoraphobia; and that the slight height of the disk from which the animal would not jump at all is due, not to any visual short-sightedness, but to the fear of jumping in general. Undoubtedly both of these conditions may obtain in the mouse when on the disk, yet the decreasing series of times elapsing before jumping, as recorded, corresponds in such a way with the decreasing height of the disk as to make it clear that vision does come into play in the estimating of distance. That this perception is not due to some other sense which receives the vibrations from near objects is shown by the poor results when glass was used. The animal seems to be unable either to see the glass or to be affected by any object on the other side of it, except when the object is very close to the glass. This is what we should expect, considering the apparently short range of vision of the mouse and its lack of perception of sharp visual outlines. The shiny surface of the glass is evidently connected with the estimated distance of objects on the other side. The black paper when covered with the glass appeared more glossy than the white. This doubtless made it appear less certain as a place to jump to than the white.

Turning the table, or the disk, or both, through an angle of 180 degrees, to act as a check against choice according to absolute position, changed the results only slightly.

PROBLEM 5. PERCEPTION OF THIRD DIMENSION IN OBJECTS

This problem is very nearly related to the preceding one. It has reference to the third dimensional nature of the object perceived rather than to the distance of the object from the animal. It is treated separately on account of the entirely different method and apparatus made use of.

APPARATUS: A box measuring 42 cm. x $23\frac{1}{2}$ cm. and 7 cm. deep, with openings at both ends, was used (fig. 7). At one end is attached the triangular enclosure *A*, and the other end of the box opens into the nest, *N*. Leading into the box from *A* is a narrow passageway 10 cm. long and 3.5 cm. wide. *B* and *C* are adjustable partitions which barely pass each other when moved back and

forth in the box. A scale is marked off on the floor of the box, extending from the point X at the end of the passageway to the opening into the nest.

Method: The animals are started from the enclosure A through the box toward the nest. Mice, owing to a stereotactic instinct, tend to linger in the passage and then dash quickly forth

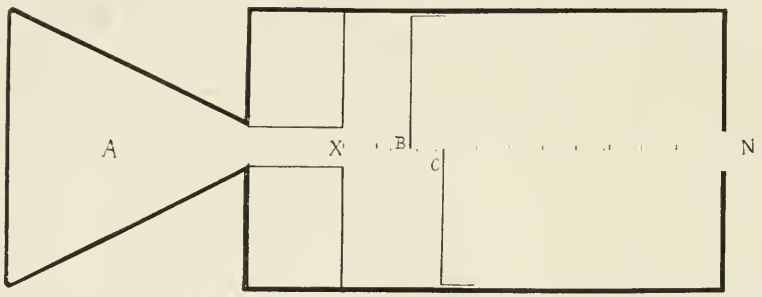

Fig. 7 Apparatus for the study of perception of depth (third dimension). A, triangular entrance chamber; X, passageway; B, C, moveable board partitions; N, nest-box.

across the open space toward the nest. In the experiment, therefore, the animal must observe from the point X which of the two partitions, B or C, is the nearer. If the partition on the left is nearer the animal, it is evident that, in order to pass through, he must swerve to the right as he runs forward, and then turn sharply to the left. If the partition on the right is the nearer, a manœuvre of the opposite sort is necessary. The mice should turn correctly in case they have perceived from X the true relative positions of the two partitions.

In the experiments food was scattered in the enclosure and the animals were allowed to run back and forth between it and the nest a few times, then while an animal was in A, the partitions were changed and a sharp clap of the hands sent him toward the nest. His behavior at the point X and at B or C was noted with care.

Thirteen mice were used.

MOUSE	COLOR	SEX	AGE	DATE OF EXPERIMENT
Ke	white	male	6 weeks	March, 1906
Kh	white	female	6 weeks	March, 1906
Ka	white	male	6 weeks	March, 1906
J	white	male	adult	March, 1906
Q	white	male	adult	March, 1906
T	gray	male	3 months	Oct. 10
X	black	female	3 months	Oct. 15
BF	black	female	3 months	Oct. 15–17
U	black	male	5 weeks	Oct. 20–25, 1906
Xa	gray and white[1]	male	2 months	Feb. 25–Mar. 23, 1907
Xc	gray	male	2 months	Feb. 25–Mar. 23, 1907
Xd	black	female	2 months	Feb. 25–Mar. 23, 1907
Xe	white	female	2 months	Feb. 25–Mar. 23, 1907

[1] The gray and white mouse had pigmented eyes.

The animals were tried separately. After each trial the positions of the partitions B and C were reversed. The reaction was recorded as an error of choice, and therefore indicative of lack of perception of depth, if the mouse inclined to the left when B was the nearer to X, or to the right when C was the nearer.

As a rule the mistakes were very marked. Often an animal would run forward until its nose touched the partition. Again an individual would slowly approach the partitions, examine them at close range, and then dash through between them. Such reactions as this last were of doubtful value in connection with the problem.

RESULTS: The following tables give the results for different groups of mice and different positions of the partitions.

Albino mice. Partitions respectively 10 cm. and 15 cm. from X. Reversed after each trial

MOUSE	NO. TRIALS	RIGHT	WRONG	DOUBTFUL
Ke	10	6	4	–
Kh	36	14	16	6
Ka	11	6	4	1
J	20	11	8	1
C	10	8	2	–

Mice with black eyes. Partitions same distance from X; changed at irregular intervals, usually after two or three trials.

MOUSE	NO. TRIALS	RIGHT	WRONG
T	10	7	3
X	14	7	7
BF	26	18	8
U	10	3	7

In the next experiment the mice T, X, BF, and U were allowed to run back and forth through the box all night. The following morning while they were in the enclosure *A*, the partitions were interchanged and they were allowed to run through the box one at a time. They were then taken back and the process was repeated. In the eight trials, seven were wrong and one right. The same thing tried on a subsequent morning resulted in eight wrong choices.

Partitions nearer to the animal, but at the same distance from each other (5 cm.) One at 5 cm. the other at 10 cm. from X

MOUSE	NO. TRIALS	RIGHT	WRONG	DOUBTFUL
Xc	20	4	10	6
Xa	14	7	5	2
Xd	18	5	11	2
Xe	15	5	8	2

One partition 5 cm. from the passageway, the other 15 cm., making a depth of 10 cm. to be perceived

MOUSE	NO. TRIALS	RIGHT	WRONG
Xc	6	1	5
Xa	6	2	4
Xd	6	2	4
Xe	8	3	5

In all of the white mice there was observed a peculiar head movement from side to side while crouching in the passageway, preparatory to running forward. Since the results in the matter of depth-perception favor the white mice—for the black-eyed

mice the number of right choices being 47 per cent of the number of trials, while for the albino mice it is 53 per cent— it was thought that this head movement might be serviceable to the animals, as giving several points of view of the object, each from a different angle, thus possibly rendering a perception of depth easier.

Following this idea, a record was kept of the number of times the head movement was observed and this was compared with the right choices. I present as typical the results in the case of one mouse, Kh. This animal made 14 right choices and 16 wrong. Of the right choices the head movement was observed in 8 and not observed in 6, while of the wrong choices there was head movement in 1 and no head movement in 15.

Conclusions: Judging by the number of errors, we may conclude that the mice do not make use of visual perception of depth. If they have the anatomical equipment necessary for the perception of depth, their important muscular sense controls their actions, making them take the same course they took on the preceding occasion.

II. BINOCULAR VISION

The question of binocular vision in the mouse suggested itself in connection with the investigation into the perception of depth, and an attempt was made to find how far the structural conditions are fulfilled which would make it possible.

The conditions which must exist in order that binocular vision in the psychological sense may be present are:

(1) It is necessary that the eyes be so situated in the head as to have a portion of the field of vision common to each.

(2) There must be consensual movements of the eyes. The lines of sight of the two eyes must be capable of moving approximately parallel to one another so that the images of an object may fall on corresponding points of the two retinae.

(3) There must be a chiasm of the optic nerve and a portion of the fibres from one eye must mingle with a portion of those from the other, that is, there must not be a total decussation of optic fibres at the chiasm.

If the animal possesses the requirements estimated above, there is good reason to suppose that it has binocular vision in the proper sense.

A piece of anatomical work undertaken to supplement the experiments on behavior was that of measuring the angle of divergence of the optic axes, and determining the angular field of possible binocular vision. The results appear in fig. 8. In a head from which the eyes had been removed the optical axis was obtained by drawing a line from the fundus of the eye-socket through

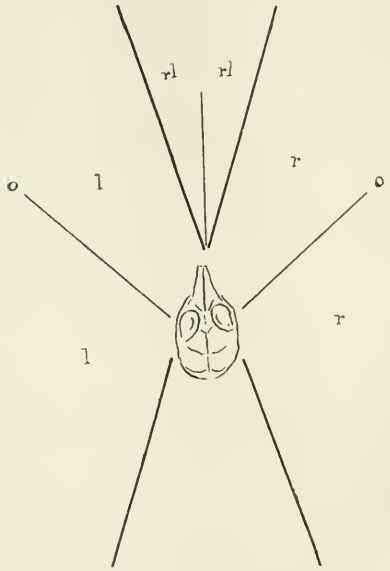

FIG. 8 The field of vision of a mouse. *r*, right monocular field; *l*, left monocular field, *rl*, binocular field; *o*, *o*, optic axes.

a point equally distant from all points in the rim of the opening, outward into space. The limits of the field of vision were determined by the points from which the eye could be plainly seen.

Although the axes of the conical eye-sockets in the mouse diverge greatly, forming an angle of about 100 degrees, yet, owing to the prominence of the eyes themselves, it is quite possible that they may receive images from the same object simultaneously.

I experimented by standing in front of the mouse where, with one eye closed, I could plainly see both eyes of the animal, and then moved my head from side to side in order to discover how great was the angular field from which the two eyes might be seen. I found that I could move my head through an angle of 70 degrees without losing sight of either eye.

Although the possibility of seeing the ball of the eye in a human being does not always mean that the eye sees the observer, yet we know that when the eye is turned as far as it can go toward the observer, he is seen on the extreme border of the field of vision.

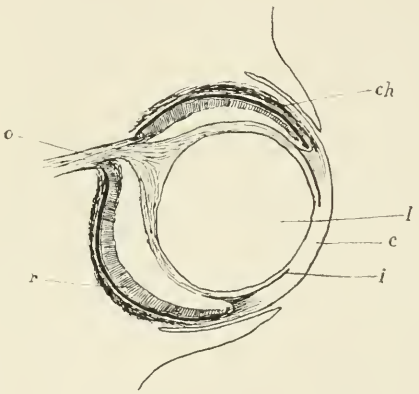

FIG. 9 Diagram showing relative size of lens in eye of mouse. *ch*, choroid; *l*, lens; *c*, cornea; *i*, iris; *o*, optic nerve; *r*, retina.

This of course, is made possible by means of the refractive power of the lens and by the fact that the retina extends all the way around the inside of the eye right up to the ciliary process.

If the retina were of uniform acuteness throughout its area, or if the lens were larger, it would not be necessary to move the eye to its limit in order to see the observer who can just see the eyeball. These are just the conditions which we have good reason to suppose exist in the mouse. In the first place the size of lens,

compared with the size of the eye, is much larger than in man (see fig. 9); and, second, the homogeneity of the retina is demonstrated in a later section (IV) of this paper.

From these considerations we feel justified in concluding that there is in the mouse a portion of the field of view which is shared by the two eyes.

Concerning the second requirement for binocular vision it is exceedingly difficult to secure data from observation of the animal. The uniform appearance of the surface of the eye makes it almost impossible to detect movements of the eye. The black glistening beadiness of the eye is always the same.[1] Several attempts were made to obtain a point of reference on the eye-ball by using a small square of paraffined Chinese white, but without success. The animal would close the eye immediately and dislodge it. Finally on close observation under a very bright light a faint line of the pupil could be distinguished, and when the light struck the eye at a certain angle, its movements could be observed.

The animal was held on the palm of the hand facing the observer and trials were made by moving a finger back and forth on different sides to learn the nature of the eye movements. When the finger was brought slowly above the level of the palm, within the field of vision for one eye, the mouse would turn its head slightly toward the finger. When the finger was moved to and fro, it was not followed by the eyes. Under these conditions the animal always reacted by remaining perfectly still with the eyes in a fixed position. A more rapid movement of the finger would elicit only a slight further turning of the head in the direction of the movement. The finger was then lowered and raised on the other side of the animal's head. These conditions were alternated and repeated about twenty times. The reaction was in all cases the same.

Fear cannot reasonably be suggested as a cause for the movements not being followed with the eyes, for the animal was perfectly tame. It was used to being handled daily and would run to

[1] It might naturally be supposed that the uniform beady appearance of the eye is of biological value in permitting the animal to make movements of the eye which, while enabling it to see its enemies, would themselves remain undetected.

my hand at any time. Further, the fact that the head was moved would make the view that fear inhibited the eye-movements untenable.

Such slight eye-movements as do occur seem to be rather for the purpose of getting more light from the general direction of the object than for getting a clearer image. There appears to be nothing in the vision of the mouse which compares with fixation in the human being. If the image of the object is cast upon any part of the retina, all the conditions are fulfilled which make vision useful to the mouse as a protective sense.

On the third requirement for binocular vision, the dividing and crossing of the optic fibres, positive statements can not be made from a mere study of the anatomical structure. The chiasm exists in all vertebrates, while in mammals, birds and to some extent, in reptiles the nerves unite, so that each is made up of fibres from both roots. In fishes they cross without uniting. Whether fibres from corresponding points in the retinae unite so that their excitations are carried to the same neural channels in the brain is practically undiscoverable, but the fact that there is any union at all rather than total decussation, indicates an intimate degree of relation between the two eyes.

Harris,[2] who worked by the method of degeneration upon the optic fibres of the lizard, ventures the statement that decussation in rats and mice is complete. More satisfactory knowledge on this point would be gained from the cumulative evidence of the degeneration method and the method of myelogenesis as used by von Gudden. The amount of mingling of optic fibres would not be great considering the fact that only small portions of the retinae can receive images from the same object. Cajal[3] working by the method of degeneration upon the optic chiasm of the rabbit and the mouse, has in fact shown that the crossing of the fibres is not complete.

[2] Harris, W. Binocular and stereoscopic vision in man and the other vertebrates, with its relation to the decussation of the optic nerves, the ocular movements and the pupil light-reflex. *Brain*, part cv, p. 107. 1904.

[3] Ramón y Cajal, S. Textura del sistema nervioso del hombre y de los verdebrados, vol. 2, p. 652, *Madrid*, 1904.

If there were no mingling at all of optic fibres, then we should be urged to the absurd conclusion that a single object which casts double images in the eyes is not interpreted as one, or else that there is an alternation of attention, a sort of psychical rivalry in which the sensation from one eye intermittently inhibits that from the other, a view which is not in accordance with the law of parsimony and is most improbable. As a third possibility it might be claimed that one of the images becomes the dominating stimulus while the sensation from the other is entirely inhibited. We find such a possibility proposed as a theory by Morgan.[4] He supposes, in the case of animals like the rabbit, where the eyes are so situated that they cannot combine in binocular vision, that "the image that falls on the most sensitive area or yellow spot of one eye suggests the focal impression, while that which falls on a similar spot in the other eye is marginal to its conscious consentience." The existence of such a yellow spot Morgan assumes. The need of a theory of this sort might be vindicated, were it shown that animals whose eyes diverge at so great an angle possess a fovea. Schäfer[5] states that only man and some primates have optic axes capable of convergence and a single central fovea.

The theory proposed above would be adequate for the explanation of the conditions which obtain in those animals, which in attending to an object turn one side of the head toward it, thus inhibiting any sensation from it by way of the other eye by practically excluding it from the other visual field, as do most birds. The mouse, however, turns toward an object enough for it to be clearly perceivable that lines from the object strike both eyes with a generous margin.

In reasoning from binocular vision in man to that possible in the mouse extreme caution is necessary, because in man many of the phenomena of binocular vision are closely connected with the central point of most acute vision in each retina. Convergence is meaningless unless we have reference to some definite point in

[4] Morgan, C. Lloyd. Introduction to Comparative Psychology. chap. 10, p. 160, *London*, 1902.

[5] Schäfer, A. E. Text-book of Physiology. vol. 2, p. 1148, 1900.

the retina, where the lines of sight which converge on the object terminate.

Although it is true that corresponding points function most accurately in the region of the central spot, as is shown by the difficulties attending the experimental determination of the horopter, yet it need not be true that they owe their existence to the presence of a central spot. The existence of corresponding points is quite possible in a retina, all portions of which are similarly organized.

The mouse, which has no fovea, might have certain portions of the eye adapted for binocular vision. These are the extreme posterior areas of the retina, which correspond to the temporal segments in the human being. It is here that images from one object can fall on both retinae and, therefore, here corresponding points must have been developed, otherwise the animal would perceive objects double.

The conditions here differ from those obtaining in the human being only in degree. In the latter a considerable portion of the nasal area of each retina can not function in binocular vision on account of the prominence of the bridge of the nose, and therefore a point in this region can have no point in the temporal portion of the other retina corresponding to it. In the mouse this area is much more extensive. If, for convenience, we call this area the monocular area, as distinguished from that in which corresponding points exist—the binocular area, then in an animal like the mouse the centre of the retina lies in this monocular area.

If there were a point of clearest vision near the center of the eye, it would be merely a fixation point which might function in lenticular accommodation. However there is no structural sign of a fovea in this region and obviously such a point in the monocular area could not function in convergence. We must conclude that the optical axis of the mouse's eye has no functional significance.

If we expected to find a fovea which serves the mouse as ours serves us, we would look for it in the extreme posterior portion of the retina, in the binocular area.

I made a number of observations upon squirrels in connection with this problem. In the squirrel the position of the eyes is some-

what less favorable to binocular vision than in the mouse. The snout of the mouse is proportionately longer than that of the squirrel, but the bridge between the eyes is so much lower that there is a large field of vision for two eyes above the head, which the squirrel does not possess.

When the squirrel under observation was approached from the side, he would sit on his haunches, lift up his head and show all signs of attention. When I would kneel on the ground within six feet of him and make no movement, he would remain with one side of his head toward me, using only one eye. When a movement was made by waving the hand back and forth, he would turn his head directly toward me in a position where both eyes were equally visible. This is a reaction very similar to that in the mouse.

Two explanations of this reaction suggest themselves: (1) The movement is made for the purpose of getting perspective which would aid in the perception of distance. Here convergence would be implied in the way that it occurs in human beings. (2) The head movement is made for purposes of orientation preparatory to turning the body in the direction of the stimulus and, perhaps, approaching it. The forward movements and turnings of the animal are executed with reference to a median plane, to which the precise relation of an object in space is more easily determined when the object is seen with both eyes. For determining the space relation of two objects both in the median plane, the one factor of location of images on the retina is adequate. Obviously a crawling animal like the mouse is more concerned with accuracy in its right and left movements than in movements in the vertical plane.

Of the two explanations given the latter is the more likely to prove correct. It is in accord with our experimental results in depth perception in the mouse, and further, the idea of convergence is not entirely compatible with a retina without a fovea, homogeneous throughout and of which the habits of the animal would demand only the function of communicating in a rough way the general nature of the object and its direction.

Perception of distance adequate to the animal's needs may be obtained through a synthetic correlation of retinal impressions and motor impulses of monocular accommodation.

Loeb[6] suggests that some animals may localize by means of changes in the form of objects, which must result from a marked astigmatism existing in the eyes of certain animals.

Cole[7] distinguishes four types of animal response to photic stimuli:

A. Response of eyeless forms.
B. Response of forms with direction eyes.
C. Response to size of luminous field.
D. Response to different objects in the visual field.

For our purpose we would make five types, dividing the last into two. Of these the first is to be found in those animals which perceive the presence of objects and a few general characteristics concerning them. The other is in those animals which have a distinct perception of form by means of a fovea or central spot of most acute vision. The mouse would be included under the first of these two. Under the latter would come man and the rest of the primates and some other animals such as the chameleon and certain birds of prey.

Animals of the former class, destitute of a fovea, although they may have a more delicate perception of the existence of objects in the field of view than we, yet do not see the form of objects regarded, as distinctly as we do.

A faint star is best seen with averted gaze owing to the fact that the rods are functioning and are susceptible to faint light stimuli but not to distinctness of outline. This is the case with the mouse, in whose retina cones do not exist.

The visual conditions existing in the mouse as revealed by our study of it are in accord with a view expressed by LeConte:[8] "In lower animals, especially those which are preyed upon by others, it is far more important to see well in every direction than to fix attention exclusively on one point, therefore, the advantages of exquisite microscopic distinctness of the center of the field are sacrificed for the much greater advantages of moderate distinct-

[6] Loeb, Jacques. *Arch. f. d. ges. Physiol.*, vol. 41, p. 371.

[7] Cole, L. J. An experimental study of the image-forming powers of various types of eyes. *Proc. Amer. Acad. Arts and Sciences*, vol. 42, p. 410, 1907.

[8] Le Conte, J. Sight, chap. 5, *New York*, 1881.

ness over a very wide field. The most important thing for them is a very wide field and the equal distribution of attention over every part. Hence their eyes are prominent and destitute of a central spot so that they see all parts with equal distinctness."

III. KINAESTHETIC SENSATIONS, THE GUIDE TO MOVEMENT

In the human being who is at all introspective, kinaesthetic sensations often come into the focus of consciousness, but presumably more often they do not. In the latter case, strictly speaking, they are hardly to be called sensations. They exist merely as neural modifications. Traces are retained within the nervous structure in the form of facilitated transitions across the synapses, or, of increased permeability of the neurones. These neural processes operate in controlling the actions of the body without necessarily involving consciousness at the time. Sometimes they emerge into consciousness late, as when we become aware of having had our limbs in a certain position and know that they are not now in that position. Again we may have kinaesthetic images of bodily actions we are about to perform.

Our theory of the guiding sense in the mouse may be introduced with an illustration from human psychology, the phenomenon of alternating personalities. The normal person may do many things of which he is wholly unconscious, *e.g.*, he may lay an object in a certain place and, after a while, search for it, entirely unconscious of having put it anywhere himself. Later when another personality is dominant, it may occur that the knowledge of the location of the object is present to consciousness and there is no difficulty whatever in finding it. The second personality remembers putting it in the place in which it is found. This phenomenon may be explained on the supposition that during the incumbency of the former personality, the kinaesthetic sensations from the movements of the limbs are unable to emerge into the conscious field because other psychical processes, viz., those to which the normal person is attending, have control of the system of neurones whose excitement is accompanied by consciousness. Association of the kinaesthetic sensations in question with the present perceptions is

inhibited by the draining of the nervous energy of the association neurones of the higher senses in the direction of the frontal tracts of the brain. Now under these circumstances, it is evident, something must become of the nervous currents coming from the muscular sense organ. They seize upon certain motor neurones of the Rolandic area whose connection with the systems operating to reinforce the higher functions is not so direct, and consequently are not so thoroughly drained of energy. Such systems are mainly those which have functioned in bringing about the movements which gave rise to the kinaesthetic sensations. In them the resistance is low. Thus it comes about that motor circuits of the second level are formed. The passing of the synapses in these circuits is rendered progressively easier on account of the reverberating of impulses through the kinaesthetic-motor system. This system and the higher systems involving attention are for the time being mutually inhibitive.

The animal in choosing between alternatives is guided mainly by kinaesthetic sensations which have been registered in the nervous system, just as is the case with the subconscious personality. When the animal enters a compartment where it must choose between situation A on the right and situation B on the left, two internal factors tend to determine action. One of these is visual, the other is kinaesthetic, Of these two the visual is the one emphasized by Thorndike in his experiments with cats. Observation indicates that in the mouse, the visual stimuli are not of so great importance in guiding the animal as the kinaesthetic. The latter is relied upon wherever possible. Smell seems to be the next in importance. These considerations prompt us to adopt a law of parsimony in studying the senses of the mouse: we are warranted in inferring a case of visual determination only when there is no possibility of the muscular sense being used for the discrimination in question. Training a mouse to discriminate always involves training him away from a reliance on the muscular sense. This law may be applied to the muscular sense and smell, or, to smell and vision. There is a suggestion here of a possible criterion for grading the intelligence of the animal series, viz., the relative importance of the various senses in directing movement.

When Thorndike put his cats into a cage, the process of learning to open the latch consisted in the gradual association of a certain movement with certain sense impressions under certain conditions of hunger. Under the instinctive excitement caused by the situation the cat makes many movements; those in each part of the cage are guided by the visual impressions of that part of the cage acting by way of the visual cortex. Each group of visual impressions would thereafter, in accordance with the law of neural habit, tend to lead to the same movements more readily than before. One of these impressions acquires increased intimacy of association with a certain movement more readily and certainly than the rest, viz., the visual impression made by that part of the cage in which the latch is situated, with that movement which results in the falling open of the door. The intimacy of this particular association is increased each time this particular movement is made, until, as the cat casts its eye over the cage, the visual impression of that part of the cage at once evokes the movement. The explanation which Thorndike gives as to why the association between the particular sensory path and the particular motor disposition becomes fixed while other possible motor dispositions do not become fixed, is, that the former association gets "stamped in" by the pleasure resulting from it, while the other is "stamped out" by the pain of failure.

The kinaesthetic sense undoubtedly has a tendency to determine the cat's behavior, but vision operates more quickly, for the cat directs its attention to the visual stimuli, rendering possible readier association between the object seen and the motor mechanism. The condition here differs only in degree, not in kind, from that obtaining in the mouse.

The relative importance of muscular association and visual association may be well shown by the analysis of the actions of a mouse used in problem 2, B. If we go on the assumption that the mouse's action was associated with the result of the action immediately preceding, then we divide the whole series of choices of the animal into two kinds of sequences: *position sequences,* in which the mouse turns to the same side, left or right, where it received food in the preceding trial, and avoids the side where it received

a shock, and *color sequences,* in which the animal goes to the color where it received food in the preceding trial and avoids the one where it received the shock. The turnings to left or right were recorded in all the experiments, and in the case of mouse Q, in 100 choices we have the following results:

Position sequences	69
Color sequences	53
Position sequences in opposition to color	26
Color sequences in opposition to position	10

We interpret the behavior of the animal thus: it enters the compartment where a choice is necessary. Its attention may be on the idea of the food which it expects to receive or upon the pain of the electric shock or anything else you please—if we admit the possibility of such attention in the mouse—but this attentive consciousness is not directive unless it is associated with the idea of moving toward the food or away from the shock, and not then unless this idea is accompanied by the actual movements, at least in their incipiency. To put it in physiological terms, there must be a connection between cortical centers for representation and the motor areas, and this must be sufficiently energized to drain the energy from the kinaesthetic system. This, as we have been led to conclude from observing the behavior of the animal, is not generally the case. The governing of movements is turned over to the motor circuits, and thus it happens that time after time the mouse runs into the same compartment, the compartment in which it may receive a shock, but still the compartment which it entered on previous occasions sufficiently numerous for a motor circuit of a certain degree of stability to become established.

The reverberating of excitation through the motor circuit in the animal may be likened to a fly-wheel, which carries the animal in one direction or another by its momentum. The cue for the revolving of the wheel is afforded by the sensations which are constantly coming from the incipient movements of the animal. These may be reinforced by tactual impressions received from the floor and walls of the compartment.

The mental state of the mouse on entering the puzzle box may

be conceived as similar to that in which one finds one's self when learning to operate some little mechanical device:

I go to my locker after a long absence, having forgotten the combination, and as soon as the muscular sensations come in from handling the lock, I find that I am turning the knob to the particular succession of numbers which will open the lock. I become aware of the succession and by attending to the movements I am making, learn the combination from myself. In this case I have been observing the effects of the operation of a motor circuit.

Supposing I am not particularly anxious to relearn the combination; my attention is on the paraphernalia within the locker. It is possible that I may open the lock without learning the combination from myself and may come to the locker again the next day without an idea of the key. Instead of being wholly interested in what I seek, it is only when I fix my attention on the means of attaining it that I am in a position to learn; but I can accomplish my ends perfectly without paying attention to the means, letting the motor circuits do the work, and this is the way, we conceive, that the mouse does in the majority of cases where it is successful.

Now suppose that after I have become used to a certain combination and can work it unconsciously, the combination is changed and I am told a new one. I receive the new series of numbers and commit it to memory. The next day I go to the locker and find, after working with the knob for some time, that my fingers have been using the old combination. I may even make this same mistake for several days. This experience we may compare with that of the mouse when the lights are changed. The creature is guided by the effects of the motor circuits within its body to the right side, because it had become habituated to turning to the right when the food was to be obtained on that side. Now that the food is to be obtained on the left, it still goes to the right, and does so over and over again. Each attempt of this sort is of course recorded as a failure.

How long the animal will continue to run into the wrong compartment is a question of how long before it will begin to attend to the means to be employed in obtaining the food, or, it is a question of how long before the impulse to venture in search of food

will be overcome by the impulse to avoid the unpleasantness of the shock which ensues in case of failure, so that the animal becomes unwilling to venture at all.

In the case of the human being, if several repetitions are necessary to bring into the focus of consciousness the movements that that being is making, then we must not expect much of the mouse, and we must believe that a large number of the cases recorded as failures were not necessarily failures to discriminate between stimuli but, rather, secondarily automatic movements.

When a visual stimulus succeeds in calling forth a change in mode of action as its response—thus overcoming the kinaesthetic influences, which would tend to bring about a former mode of action—there is involved a discharge of energy through the higher association tracts and a conscious accompaniment. The law of parsimony proposed at the beginning of this section, and also our general observations, forbid us to assume that such conscious accompaniment is involved, but rather make for the view that the mental processes of the animal rise into consciousness of its movements at intervals not so frequent as in the human being, and that the kinaesthetic sense is the predominant directive sense in the mouse.

IV. STRUCTURE OF THE EYE OF THE MOUSE

The animals used for the anatomical study of the eye were from among those that had been used in the experiments. Some gray mice were chosen and some albinos; also a few dancing mice were included among those studied.

Method: The heads of mice that had recently died or had been killed were preserved in formol-alcohol. When ready to work with them, the eyes were removed and put through alcohols of increasing strength, for about an hour in each, up to 100 per cent. They were then transferred to xylol where they cleared over night and the next day they were put in soft paraffin for an hour. They were next placed in hard paraffin for a half hour and later imbedded in this same paraffin. Care was taken in the imbedding

that the exact orientation of the eye, dorsal-ventral and anterior-posterior, should be known and preserved as the paraffin hardened.

In those eyes to be used for the study of the retina it was found necessary to remove the lens because, being made harder by the alcohol than the rest of the eye, the knife in striking it would have a tendency to tear the more delicate retina. The lenses were removed best after the eye was in the hard paraffin. A knife was passed through the block just cutting off the cornea, the lens was then easily picked out with a needle and the whole block was reimbedded. In other eyes the lenses were removed while still in xylol.

The paraffin blocks were next cut by the microtome into sections ten micra in thickness. These were taken in serial order and mounted by the water method upon slides smeared with albumen. The slides were warmed, washed in xylol and plunged into absolute alcohol, after which they were run through alcohols of decreasing strength in order to use an aqueous stain. They were then stained in Delafield's hematoxylin for three hours, washed in water, then counterstained over night in orange-G solution. After having been removed from the last solution they were run up through the alcohols again, immersed in xylol and finally mounted in Canada balsam.

Results: A diligent study was made of each series to learn the nature of the retinal elements. There was no evidence of cones to be found, either in those sections which had been cut from the dorsal side downwards, exhibiting horizontal sections of the retina at the middle of the eye, or in the first sections of the eye, where the elements would appear in cross section (fig. 10).

No cones were visible among the retinal elements in any section of the eye. There were among the rods certain larger appearances which were at first thought to be cones, but it was found that these were caused by the overlapping of some of the rods or by their separation from one another by an interval a little greater than usual. They were not cones, as they did not take any stain and in some cases they tapered away at both ends.

Some of the eyes were cut beginning with the fundus outwards. In the first few sections of the series cut in this way, where one

would expect to find cones if anywhere, there was no sign of a cross section of a cone.

The best presentation of the retina of the mouse can be given by a diagram (fig. 10). Fig 9 is a drawing of a section through the whole eye, showing the size of the lens in comparison with the rest of the eye.

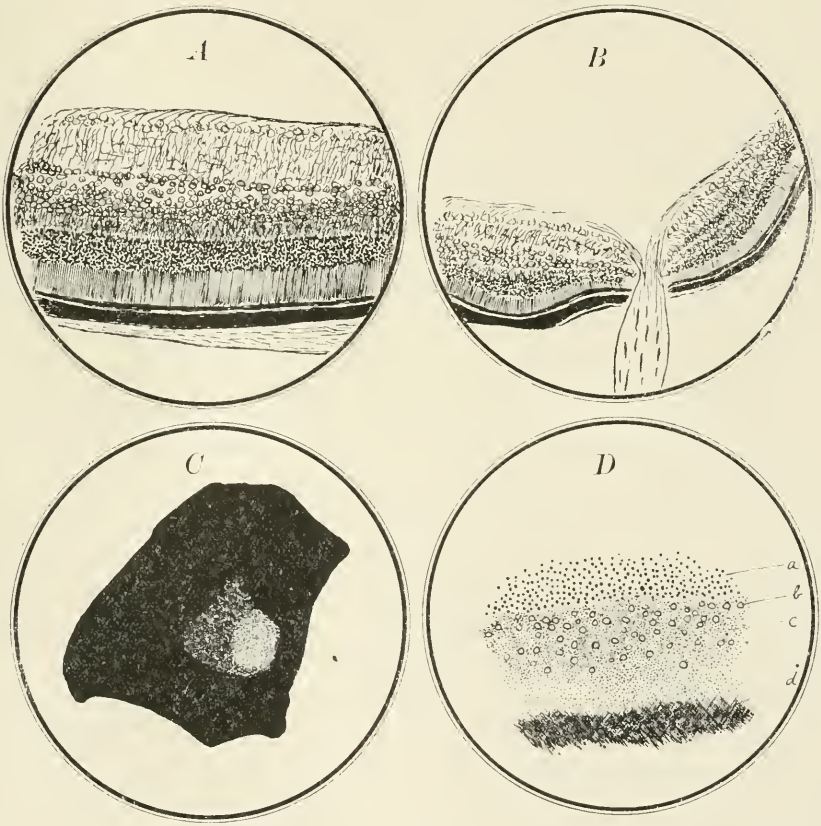

FIG. 10 A Section from central region of retina of mouse, showing rods without admixture of cones.

B Retina of mouse, showing entrance of optic nerve.

C First section of retina of eye of mouse, dorsal cutting, showing choroid coat, pigment cells and rods, no cones being present.

D Section of retina of eye of rabbit, showing rods and cones. *a*, nuclear layer; *b*, cones; *c*, rods; *d*, pigment.

A search through the sections of the eye failed to reveal structural signs of a fovea in any part of the eye. There was to be found no point in which the inner layers of the retina are sacrificed for the benefit of the rod layer, nor was there discovered a differentiation of the rods in any particular region.

V. SUMMARY

We may now bring together for convenience of reference the conclusions that have been drawn from the several researches:

1. The mouse distinguishes differences in grays and in brightness of lights with a considerable degree of accuracy. The discrimination of the albino mouse is not so good as that of the mouse with pigmented eyes. Illumination of the environment is influential in determining choice of light or dark objects. Black and white are preferred to grays.

2. Red and blue objects, which appear of the same intensity to the human eye, are discriminated between by the black mice, the percentage of error being less than in the case of the grays. Red and yellow are preferred to blue and green.

3. Albino mice do not show any discrimination between red and white lights. With black mice a very bright red and a white of low intensity are distinguished with greater difficulty than colors which are to the human eye of equal brightness. Discrimination between green and blue light is not apparent.

4. Perception of form is very poorly developed. The eye does not seem to be suited to any distinct perception of outlines.

5. The distance of objects is perceived within a range of 15 cm.

6. The mice fail to profit by estimating the depth of objects. The black mice make more errors in this respect than the albinos.

7. Our anatomical investigation shows mice to be lacking in retinal cones, confirming what has been surmised by Allen and by Morgan, and stated by Slonaker. We do not think it follows, as Morgan would suggest, that the absence of cones in mice, bats, hedgehogs and such nocturnal animals implies inability to distinguish colors. It is quite possible that the rods in the mouse are

adapted to the distinguishing of such few color contrasts as may be of importance in its life and habits.

8. There is no fovea or other structurally differentiated portion in the eye of the mouse. The range of vision is very wide, all parts of the retina being equally sensitive, a condition which is enjoyed at the sacrifice of distinct perception of form.

9. There is possible for the mouse a small field of binocular vision. This is not used for estimating distance, as there is no convergence of the eyes. It is of service rather as a means of orientation.

10. The kinaesthetic sense is more important than vision in determining the actions of the mouse. The latter is of use in indicating the presence and general direction of an enemy. Food is found largely through the sense of smell. In other words, smell is an active sense; vision is a protective or passive sense, while the behavior of the animal is largely the result of motor habits, formed through kinaesthetic sensations.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. NO. 212.

REACTIONS OF FROGS TO CHLORIDES OF AMMONIUM, POTASSIUM, SODIUM, AND LITHIUM

LAWRENCE W. COLE

The experiments herein recorded were designed to test reactions of the common leopard frog, Rana pipiens Schreber, to solutions of the chlorides of ammonium, potassium, sodium, and lithium. As these reactions were obtained from frogs whose brains had been destroyed, they are, strictly speaking, spinal-cord reactions and dependent, therefore, on spinal nerves. Whether or not they can be related to taste will be discussed toward the close of this paper. The subject was suggested to me by Professor G. H. Parker and the work was done under his direction.

A series of preliminary experiments showed that the lower limit of the susceptibility of the frog's skin to these salts is not far from a solution of m./2 strength. Even the most responsive specimens of R. pipiens reacted only once or twice to m./2 solutions of the chlorides of sodium or of lithium. They reacted very frequently, however, to ammonium and potassium solutions of that strength. 3 m. solutions were the strongest ones tried, for larger amounts of some of these chlorides do not dissolve completely in water.

Merck's salts were used in the preparation of the solutions and each solution was titrated against one of potassium chloride taken as a standard until results within 2 per cent of accuracy were obtained. The titration was done in silver nitrate with potassium chromate as an indicator.

Freshly prepared brainless frogs were suspended by the lower jaw from a hook on a lever apparatus such as had already been used by Parker and Metcalf ('06) for similar experiments on earthworms. Essentially the same method had previously been em-

ployed on frogs by Loeb ('02), and by Braeuning ('04). After suspension both hind feet of the frog were dipped into a given solution up to the ankle, care being taken to avoid contact with the sides of the containing vessel. A frog whose feet were thus dipped would withdraw them after they had been in the solution a few seconds, and this period, while variable, is significant for the particular solution. These periods usually became longer as the trials proceeded, a condition due probably more to the gradual death of the animal's tissues than to the direct effects of the salts.

A frog dipped in this way in ordinary tap-water or distilled water did not withdraw the feet at all. The time from the moment of immersion to the moment of withdrawal was taken in seconds and fifths of a second by means of a stop-watch. This time, which may be called the reaction-time,[1] is much shortened when there is an abrasion of the skin on the frog's foot. In such cases the animal responds quickly with the abraded foot and often much more slowly with the uninjured one. Fatigue effects were eliminated by returning each frog to a vessel of water during the time required to test three others, and as the four salts were tried in a different sequence in each one of every series of four animals, the order of procedure could have no effect on the averages of the reaction-times. For example, in table 1, the first frog (A) was dipped successively in potassium, sodium, ammonium, and lithium; the second (B) in sodium, ammonium, lithium, and potassium; the third (C) in ammonium, lithium, potassium, and sodium and so on. Hence the average reaction-time for any one salt cannot have been influenced by the position of that salt in the series of tests. The experiments were made at a uniform room temperature.

The reaction-times were recorded as in table 1; each number here being the record of one trial and each frog in order being tried

[1] From the behavior of frogs with abrasions of the skin, I believe that the reaction-time proper is but a small part of the total time which elapses between immersion and withdrawal. The major part of this time seems to be that required for the solution to pass far enough through the skin to meet the nerve-endings. The skin seems to present a considerable obstacle to this passage.

TABLE 1

Reaction-times in seconds of four frogs (A-D) to chlorides of potassium, sodium, ammonium, and lithium. Strength of solutions, 3 m. Date: April 13.

Frog A

Salts	KCl	NaCl	NH_4Cl	LiCl
Trials				
1	2.2	1.2	1.2	2.6
2	1.4	1.8	1.4	3.0
3	2.0	3.2	2.0	5.2
4	2.0	6.2	3.8	N.R.
5	N.R.	N.R.	N.R.	N.R.
Averages	1.9[2]	3.1	2.1	3.6
	+1 N.R.	+1 N.R.	+1 N.R.	+2 N.R.

Frog B

Salts	NaCl	NH_4Cl	LiCl	KCl
Trials				
1	1.4	0.8	1.2	1.2
2	1.2	2.0	3.0	1.8
3	2.4	2.0	3.0	1.8
4	2.6	1.8	5.0	3.0
5	4.8	3.2	4.0	3.6
Averages	2.48	1.96	3.24	2.28

Frog C

Salts	NH_4Cl	LiCl	KCl	NaCl
Trials				
1	1.4	2.0	2.0	1.6
2	1.2	1.8	1.8	2.6
3	1.8	3.2	2.6	3.0
4	2.0	5.6	2.8	4.0
5	3.0	12.8	2.8	N.R.
Averages	1.88	5.08	2.4	2.8
				+ 1 N.R.

Frog D

Salts	LiCl	KCl	NaCl	NH_4Cl
Trials				
1	1.2	1.2	1.2	1.8
2	1.8	2.0	2.2	1.4
3	3.6	2.4	3.0	2.4
4	6.0	2.0	6.0	3.4
5	N.R.	N.R.	N.R.	N.R.
Averages	3.15	1.9	3.1	2.25
	+ 1 N.R.	+ 1 N.R.	+ 1 N.R.	+ 1 N.R.

[2] Only the actual number of reactions is used in computing the averages.

in each of the four solutions. Ordinarily five records were obtained from each for each salt, though occasionally a frog ceased to react before the required twenty records were completed. Each frog was given two minutes in which to respond. A failure to react within that time was called "no reaction" and is indicated by the letters N.R. in the tables. The experiments were begun early in April and as the season advanced the frogs gained in activity, so that more than twenty records were readily obtainable in experiments made in the latter part of April, in May, and in early June. But when the weather became warm in June, it was found that the frogs again succumbed quickly to the effects of the operation. Experiments in very warm weather were, therefore, not feasible.

In examining table 1, it will be seen that ammonium chloride and potassium chloride are almost balanced in effect so far as the four frogs are concerned, for with frogs B and C ammonium produced a reaction more quickly than potassium, while with frogs A and D, potassium caused the quicker response. When, however, the general averages, from these solutions are compared, ammonium chloride (2.03 seconds and 2 "no reactions") is seen to call forth a slightly quicker reaction than potassium chloride (2.14 + seconds and 2 "no reactions"). Next to potassium chloride in quickness of the reaction called forth from all four frogs is sodium chloride with a general average of 2.84 seconds and 3 "no reactions," while lithium chloride, which has a general average of 3.82+ seconds and 3 "no reactions," is slowest. Thus 3 m. solutions of these four salts are not equally stimulating, but they form a series, which, passing from the most stimulating to the least, is ammonium, potassium, sodium, and lithium.

It will be seen from table 2 that at a concentration of 2 m. ammonium chloride again calls forth the quickest reaction, the average time being 3.64 seconds. As at concentration 3 m., ammonium is here followed in sequence by potassium (4.34 seconds), sodium (15.54 seconds), and lithium (18.86 seconds + 1 N.R.). Moreover, at a concentration of 2 m. the four salts are clearly divided by their reaction-times into two groups—ammonium and potassium; sodium and lithium—a condition suggested in

TABLE 2

Reaction-times in seconds of four frogs (E–H) to chlorides of potassium, sodium, ammonium, and lithium. Strength of solutions, 2 m. Date: May 2.

Frog E

Salts	KCl	NaCl	NH_4Cl	LiCl
Trials				
1	8.2	14.2	4.6	10.2
2	6.4	8.0	5.2	12.4
3	6.2	13.2	8.2	20.6
4	9.0	20.6	9.4	17.2
5	6.4	18.4	8.0	21.4
Averages	7.24	14.88	7.08	16.36

Frog F

Salts	NaCl	NH_4Cl	LiCl	KCl
Trials				
1	4.6	2.8	8.4	2.0
2	12.8	2.6	12.6	3.2
3	15.2	3.6	17.2	3.4
4	18.0	2.2	30.0	3.0
5	21.2	2.2	26.2	3.8
Averages	14.36	2.68	18.88	3.08

Frog G

Salts	NH_4Cl	LiCl	KCl	NaCl
Trials				
1	2.0	8.2	3.6	10.0
2	1.6	21.4	3.0	18.2
3	2.8	20.2	3.4	26.6
4	3.6	34.6	7.0	27.0
5	3.8	37.6	8.2	31.2
Averages	2.76	24.40	5.04	22.60

Frog H

Salts	LiCl	KCl	NaCl	NH_4Cl
Trials				
1	5.2	1.6	5.0	1.6
2	16.2	1.4	7.2	2.0
3	N.R.	2.0	11.2	1.8
4	20.2	2.6	13.8	2.2
5	18.6	2.4	14.4	2.6
Averages	15.05 + 1 N.R.	2.00	10.32	2.40

TABLE 3

Reaction-times in seconds of four frogs (I-L) to chlorides of potassium, sodium, ammonium, and lithium. Strength of solutions, 1 m. Date: April 27.

Frog I

Salts	KCl	NaCl	NH_4Cl	LiCl
Trials				
1	1.4	1.6	2.2	7.2
2	1.2	2.2	2.0	11.0
3	2.0	4.0	3.8	1.8
4	1.4	2.2	2.0	6.0
5	1.4	4.8	2.4	10.0
Averages	1.48	2.96	2.48	7.2

Frog J

Salts	NaCl	NH_4Cl	LiCl	KCl
Trials				
1	5.8	9.4	10.4	5.4
2	16.2	8.0	24.2	4.4
3	26.0	5.2	23.8	2.2
4	12.2	2.2	24.6	2.0
5	11.8	5.2	27.0	1.8
Averages	14.4	6.0	22.0	3.16

Frog K

Salts	NH_4Cl	LiCl	KCl	NaCl
Trials				
1	1.6	5.2	1.6	2.2
2	2.6	3.0	1.2	3.6
3	3.2	11.8	1.6	2.6
4	2.6	8.8	1.4	5.0
5	2.2	9.2	1.6	5.2
Averages	2.44	7.6	1.48	3.72

Frog L

Salts	LiCl	KCl	NaCl	NH_4Cl
Trials				
1	2.0	1.2	1.6	1.4
2	3.0	1.2	1.4	2.0
3	4.4	1.6	2.6	2.4
4	2.2	1.6	2.8	3.0
5	4.0	1.6	4.4	2.8
Averages	3.12	1.44	2.56	2.32

table 1, in that ammonium and potassium at least fall close together in their reaction-times.

Table 3 shows that with all four frogs (I–L) potassium chloride was more stimulating than ammonium chloride; the former is represented by an average reaction-time of 1.84 seconds, the latter by one of 3.31 seconds. In other respects the sequence of the salts is the same as that shown in table 2, in that the two remaining salts follow those just mentioned in the order sodium (5.91 seconds) and lithium (9.98 seconds). Not all determinations at 1 m. concentration were as uniform as those shown in table 3. Thus, in a second series of tests at this concentration much irregularity was found. This is shown in the records of this series in table 4.

Notwithstanding the irregularities in table 4, the general averages show the same sequence as in table 3. Thus potassium chloride has the shortest reaction-time (6.62 seconds), and this is followed in sequence by ammonium (10.80 seconds + 1 N.R.) sodium (34.43 seconds + 3 N.R.), and lithium (46.97 seconds + 6 N.R.). In this table, moreover, the grouping characteristic of table 2 also occurs; namely, potassium is paired with ammonium and sodium with lithium.

The weakest solutions of the salts tested were m./2 and the reactions at this concentration are given in table 5.

From table 5 no reliable averages can be computed because of the large number of failures to react. It will be seen that these records of "no reactions" are most numerous for sodium (16) and lithium (17), while potassium shows none and ammonium six. Judged from the standpoint of the numbers of failures to react, the four salts, beginning with the most stimulating, form the same series as that seen at concentration 1 m.: namely, potassium, ammonium, sodium, and lithium. It is also to be noticed that they fall again into two groups—potassium and ammonium: sodium and lithium.

As a summary of the experiments thus far described, the average reaction-times, etc., for the four salts at the various concentrations used are given in table 6.

TABLE 4

Reaction-times in seconds of four frogs (M-P) to chlorides of potassium, sodium, ammonium, and lithium. Strength of solutions, 1 m. Date: April 23.

Frog M

Salts	KCl	NaCl	NH_4Cl	LiCl
Trials				
1	1.6	N.R.	N.R.	N.R.
2	53.8	N.R.	29.2	51.2
3	1.8	54.0	5.4	34.8
4	5.0	48.4	9.0	36.0
5	8.0	34.6	4.4	34.0
Averages	14.04	45.6 + 2 N.R.	12.0 + 1 N.R.	39.0 + 1 N.R.

Frog N

Salts	NaCl	NH_4Cl	LiCl	KCl
Trials				
1	5.0	3.0	5.6	2.4
2	4.8	3.4	N.R.	3.2
3	N.R.	11.2	N.R.	7.4
4	5.4	3.2	N.R.	3.8
5	3.2	2.8	N.R.	3.0
Averages	4.6 + 1 N.R.	4.72	5.6 + 4 N.R.	3.96

Frog O

Salts	NH_4Cl	LiCl	KCl	NaCl
Trials				
1	12.8	19.0	5.0	15.2
2	19.6	33.6	3.0	16.2
3	27.8	61.4	3.6	18.4
4	17.0	57.2	3.2	13.4
5	16.0	74.0	3.8	23.6
Averages	18.64	49.04	3.72	17.36

Frog P

Salts	LiCl	KCl	NaCl	NH_4Cl
Trials				
1	26.8	3.8	64.0	15.2
2	N.R.	5.6	69.4	6.2
3	68.2	5.4	91.0	7.6
4	88.0	5.6	55.0	6.4
5	67.8	3.4	63.8	5.0
Averages	62.7 + 1 N.R.	4.76	68.64	8.08

TABLE 5

Reaction-times in seconds of four frogs (Q-T) to chlorides of potassium, sodium, ammonium and lithium. Strength of solution, m./2. Date: May 3.

Frog Q

Salts	KCl	NaCl	NH_4Cl	LiCl
Trials				
1	1.2	3.8	2.2	9.4
2	1.6	4.8	5.4	N.R.
3	3.8	N.R.	N.R.	N.R.
4	3.8	N.R.	N.R.	N.R.
5	4.2	N.R.	N.R.	N.R.
Averages		3 N.R.	3 N.R.	4 N.R.

Frog R

Salts	NaCl	NH_4Cl	LiCl	KCl
Trials				
1	3.2	4.00	N.R.	4.8
2	N.R.	161.8	N.R.	4.8
3	N.R.	84.8	N.R.	4.6
4	N.R.	47.8	N.R.	3.8
5	N.R.	70.8	N.R.	5.0
Averages	4 N.R.		5 N.R.	

Frog S

Salts	NH_4Cl	LiCl	KCl	NaCl
Trials				
1	1.8	N.R.	2.8	N.R.
2	9.8	N.R.	8.4	N.R.
3	N.R.	N.R.	3.4	N.R.
4	N.R.	N.R.	5.8	N.R.
5	N.R.	N.R.	12.0	N.R.
Averages	3 N.R.	5N.R.		5 N.R.

Frog T

Salts	LiCl	KCl	NaCl	NH_4Cl
Trials				
1	11.2	1.2	68.8	16.4
2	84.4	4.8	N.R.	65.8
3	N.R.	4.6	N.R.	54.2
4	N.R.	6.0	N.R.	75.2
5	N.R.	5.6	N.R.	68.8
Averages	3 N.R.		4 N.R.	

TABLE 6

Average reaction-times in seconds to solutions of chlorides of ammonium, potassium, sodium, and lithium in concentrations 3m., 2m., 1m., and m. 2. (See tables 1, 2, 3, and 5.)

Salts		NH_4Cl	KCl	NaC.	LiCl
Concentrations	3 m.	2.03+2 N.R.	2.14 + 2 N.R.	2.84 + 3 N.R.	3.82 + 3 N.R.
	2 m.	3.64	4.34	15.54	18.86+ 1 N.R.
	1 m.	3.31	1.89	5.91	9.98
	m./2.	6 N.R.	0 N.R.	16 N.R.	17 N.R.

TABLE 7

Reaction-times in seconds of frog V to 3 m., 2 m., 1 m., and m./2 solutions of sodium chloride. Date: April 12.

Concentrations...	3 m.	2 m.	1 m.	m./2.
Trials				
1	1.6	5.2	18.4	67.2
2	1.4	11.0	15.6	N.R.
3	2.0	15.6	22.2	N.R.
4	2.8	14.2	N.R.	90.8
5	2.4	18.4	20.8	N.R.
Averages.....	2.04	12.88	19.25 + 1 N.R.	79.0 + 3 N.R.

TABLE 8

Degree of dissociation per mill of the chlorides of ammonium, potassium, sodium, and lithium in aqueous solutions of 3 m., 2 m., 1 m., m./2 concentrations. (Calculated from Kohlrausch und Holborn, '98.)

Salts		NH_4Cl	KCl	NaCl	LiCl
Concentrations	3 m.	677	673	512	446
	2 m.	707	705	587	523
	1 m.	744	748	674	623
	m./2.	779	779	733	697

It will be seen from table 6 that the four salts, as already pointed out in discussing the results of the individual tables, tend to fall into two groups—ammonium and potassium with relatively short reaction-times; sodium and lithium with relatively long ones. It will also be seen that lithium is always less stimulating than sodium, likewise that in the stronger concentration (3 m., 2 m.)

potassium is less stimulating than ammonium, though in the weaker concentrations (1 m., m./2) the reverse is true.

It is also evident in comparing the various averages in table 6 that individual differences in the groups of frogs must be taken into account in interpreting the table. It is to be expected that the reaction-time would shorten with increased concentration of the stimulating solution and this expectation is realized in all parts of the table except those referring to concentration 1 m. Here the reaction-times are shorter than might have been anticipated, but this condition is probably due, not primarily to the solutions, but to the individual peculiarities of this particular set of frogs. That frogs vary much in this respect, can be seen in many of the tables; thus, of the four frogs whose reactions are recorded in table 3, frog J is slowest in its average reaction-times for all salts, and frog L is quickest. It is, therefore, very probable that the unexpectedly short reaction-times at concentration 1 m. were due to the accidental use of four unusually rapid frogs. This opinion is supported by the results (table 7) of testing a frog with one salt at the four concentrations used. As will be seen in the table the reaction-times lengthen with increased dilution.

In seeking for an explanation of the reaction-times called forth by the different salts, one naturally turns to the ionic contents of the solutions. The degrees of ionization at the concentration of the four salts used in these experiments are given in table 8.

According to the degrees of dissociation given in table 8, the four salts experimented with fall into two groups, one consisting of ammonium and potassium, and the other of sodium and lithium. As I have already pointed out, the reaction-times (tables 1 to 5) lead independently to a like grouping of these salts, so that these times alone would enable the experimenter to distinguish these two groups, especially at the concentrations 2 m. and 1 m.

The relation, therefore, between the reaction-times and the degree of dissociation is close, though as a comparison of tables 6 and 8 will show, it is not mathematically proportionate. Braeuning ('04) has stated that in stimulation by salts the reaction-time is not primarily a function of the diffusion process, as he believes

it to be in stimulation by acids. He proposes as an explanation of stimulation by salts that the osmotic pressure may act as a stimulus and therefore that the summation of stimuli is an important factor in the reaction-time. Consequently he believes that the action of salts may be regarded as (in a sense at least) a mechanical stimulation. This view, that both the diffusion-time and summation-time are parts of the total reaction-time, would lead us not to expect a proportional relation between the reaction-time and the degree of dissociation of the salt.

Braeuning's view seems to receive support from my observations on frogs with an abraded foot, but on the other hand the fact, as will be shown presently, that cocaine interferes so little with these reactions, must be taken into consideration in determining the nature of the stimulus.

In concluding the comparison of reaction-times and degrees of dissociation, it may be said that, though there is a fairly close relation between these two factors, this relation is probably not as close as may be implied in some of the tables. Thus, at 3 m.- and 2 m.- concentration the ammonium salts are slightly more dissociated than the potassium salts, while at 1 m. the reverse is true and the reaction-times indicate a like relation. It is, however, improbable that the frogs react to these slight differences, and I believe this feature of the tables to be accidental.

It seems quite probable that the time required for diffusion is in a strict relation to the degree of dissociation and that this relation is slightly disturbed by the added factor of summation of stimuli, or nervous reaction-time.

Since chlorine is the common ion in these salt solutions and yet the results maintain a uniform series of differences in different solutions, the chlorine would seem to have either no effect upon the frogs or a uniform effect in such series of solutions of the same concentration. The differences in reaction must, therefore, be due to the effects of ammonium, potassium, sodium, and lithium ions. Kahlenberg ('98) in writing of the effects of these ions on the sense of taste, says that, "lithium ions have no pronounced taste, their effect is somewhat like that of sodium, though less in degree. Potassium ions have a more pronounced taste than sodium ions, and ammonium ions also have a bitter taste."

From these statements I deem it not improbable that the intensity of the taste sensations of these metals varies from that of lithium, which is weakest, to that of potassium or ammonium, which are strongest, almost exactly as their stimulating effects on these frogs vary. It is evident from Kahlenberg's words that the intensities of effect in the two cases are at least very similar. The solutions required in our experiments are of course much stronger than those necessary to produce taste sensations, but the range seems comparable with that of taste. The question at once suggests itself, Does the frog's skin possess a general chemical sense comparable with the special sense of taste? One naturally assumes that the stimuli which produce these definite reflexes would in normal frogs give rise to some kind of sensation. If this be so, one of two alternatives is apparently open to us. These reflexes correspond to what in the normal frog is a general chemical sense akin to taste, or else, with their definitely timed reactions, they correspond to different degrees of pain. As a test of the latter question several frogs were treated with a 1 per cent solution of cocaine until irresponsive to superficial pricking and scratching with a needle and to superficial pinching with forceps. They were then dipped as usual into a 3 m. solution of ammonium chloride. Even in those which most quickly succumbed completely to the poisoning effect of cocaine, one reaction to the chloride was obtained after complete loss of the pain reactions. Others, not so quickly poisoned, reacted for thirty to forty minutes to repeated dippings into the chloride while the pain reactions were totally absent. If, however, the entire foot was grasped and pinched in the forceps a reaction occurred showing that the deeper nerves were as yet essentially unaffected by the drug. In these tests the superficial stimulation, scratching and pricking, was severe enough to draw blood. It, therefore, seems clear that the reactions to the chloride are not pain reflexes. As the frog's skin has been shown to be sensitive to light (Parker, '03), may it not also be sensitive to chemicals in a way more analogous to taste than to any other sense with which we are acquainted or to which we may refer it in comparison?

SUMMARY

The reaction-times of frogs to 3 m., 2 m., 1 m., and m/2 solutions of the chlorides of ammonium, potassium, sodium, and lithium give good grounds for distributing these salts into two groups—ammonium and potassium; sodium and lithium, an arrangement also indicated by their degrees of dissociation.

The quickest reactions occur generally with the chloride of greatest dissociation. In two chlorides whose degree of dissociation is not widely different, the separation by reaction-times is imperfect though a long series of experiments designed to test this point might yield discriminating results.

It is probable that the reaction-times to these salts include two factors, diffusion-time and summation-time. The latter seems to be much the shorter.

The comparisons with the tastes of chlorides of these metals and the results of applying cocaine, suggest that nerves of a general chemical sense rather than pain nerves are affected by the chlorides.

In conclusion, my thanks are due to Professor G. H. Parker for the plan of the experiments and assistance in the work.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, UNDER THE DIRECTION OF E. L. MARK, DIRECTOR. No. 213.

THE MOVEMENTS OF THE EARTHWORM: A STUDY OF A NEGLECTED FACTOR[1]

SERGIUS MORGULIS

TWO FIGURES

In presenting these observations upon the movements of earthworms and the conclusions to which the results of specially arranged experiments have led me, I shall deal with two distinct problems. The one is, whether the earthworm in locomotion reacts as a succession of separate segments or as a unit-organism; the other is, what determines the worm's movement in a definite direction? Strange as it may seem at first glance, a definite solution of the first problem depends to some extent upon the solution of the second. It is, therefore, impossible to treat the problems quite independently.

Friedländer ('04), who very thoroughly investigated the mechanism of the earthworm's locomotion, maintains that in the normal creeping of the worm there is no nervous impulse passing from one end of the animal to the other, but that "every segment by its activity furnishes the stimulus that causes the adjacent segment to become active in a similar way; therefore, in quiet normal creeping the earthworm functions not as a unit-individual, but, so to speak, as a chain of segments" (p. 181).

This view, as I understand it, is in essence, a subtle analysis of the mechanism of locomotion; Friedländer finds that a certain degree of muscular extension invariably precedes a contraction, and that a segment in the state of contraction exerts sufficient pull upon the next segment to induce a general contraction in

[1] I wish to express my sincerest thanks to Professor G. H. Parker, who spared neither time nor trouble in helping me in this work with deed and criticism.

that segment. Locomotion is, accordingly, accomplished through the alternation of passive extensions and active contractions of successive segments.

To me it appears that, while in its wide aspects Friedländer's analysis is perfectly correct, it becomes, when applied to idividual segments, a matter of inference rather than of direct observation. Although I have repeated some of Friedländer's experiments, and confirmed practically all his statements, I have failed to convince myself that his hypothesis is correct. On the contrary, every observation has strengthened my opinion that the locomotion of the earthworm is reducible to the reaction not of single segments, but of whole groups of segments.

This can be readily demonstrated by the simple experiment of cutting a worm in two, and stitching the anterior and posterior pieces together with a fine silk thread. The anterior piece, crawling on a flat surface, tugs along the posterior piece, and both move by strictly coördinated movements as though they were not detached. If the tension of the thread connecting the two pieces is relaxed, however—as is the case, for instance, when the anterior piece changes its course—the movements of the posterior piece stop at once. This clearly proves that the reaction of the posterior piece is caused by the pull exerted on it by the anterior one. Now, when the posterior piece is being tugged, a part of it, comprising six or even more segments, is at first drawn out; after a certain limit of elongation has been reached, a vigorous contraction of the longitudinal muscles pulls the remaining segments forward. In the remaining segments the process observed in the first will thereupon be precisely repeated.

Other experiments were likewise performed where, instead of stitching the anterior and posterior parts of a worm together, pieces of the nerve cord alone were removed. The method of operating was different from that used by Friedländer, who was obliged to cut the worm open to get at a particular portion of the nervous system. In my own work such operations were accomplished with the least possible injury to the body-wall of the animal, by pulling out the nerve cord. For this purpose the worm was first stupefied and a ventral incision was then made extend-

ing over one or two segments. Having thus gained access to the nerve, a pair of curved forceps, or a bent needle, was brought under it, and by gently lifting the instrument sufficient force was exerted to break the frail connections with the surrounding tissue, causing the nerve to slip out from the body. By this means it was possible to extract even as many as 20 to 25 ganglia at a time; once, indeed, a portion of the ventral chain together with the cerebral ganglia was pulled out. But the method does not meet with equal success in different kinds of earthworms. In Lumbricus terrestris, for instance, where the nerve is too fragile and rigid, it cannot be pulled out, but in Allolobophora foetida the nerve is elastic and the results of the operation are therefore more or less certain.

The small wound heals over rapidly, but the "nerveless" region can be readily distinguished on account of the strong contraction of its longitudinal muscles. In fact the latter are in a state of constant and maximum contraction, because the "nerveless" region remains conspicuous for its diameter even when the adjacent normal segments have contracted. Friedländer having observed the same condition in his worms attributed the excessive swelling of the "nerveless" region to a connective substance (ein indifferentes Narbengewebe) binding the cut muscle-fibers. Since a swelling of the "nerveless" region is invaribly found also in cases where the muscle fibers have not been injured, his interpretation is apparently incorrect.

The complete retraction of the setae, which has been observed in every operated animal, is another peculiarity characteristic of the "nerveless" region of the worm.

During the first few days after the operation the application of various stimuli, such as irritation with a needle, weak alcohol or electrical stimulation, produces no effect within the "nerveless" region, but as time goes by the sensitiveness returns slightly and local responses are generally obtained upon stimulation. The secretion of mucus in the "nerveless" region is somewhat lessened, though a strong stimulus may cause an abundant secretion. Usually, however, this portion of the worm is in a condition of greater dryness than the rest of its body but, when stimulated, the exudation of mucus in the furrow between segments may be seen.

An animal with a break in its nervous system somewhere about the girdle remains, as a rule, quiet and motionless when placed upon moist filter paper, but occasionally it takes to creeping; in that case the movements of the anterior and posterior parts are completely coördinated, though the injured portion takes no active part in the movements. However, while the worm is inactive the "nerveless" portion may be touched or poked, treated with alcohol, hot water or an electric current without causing the least disturbance to the other parts of the worm. It is true that local muscular contractions may be produced by increasing the stimulus, but the impulse is not transmitted to adjacent segments which have the nerve intact. The local response of the musculature of a deficient segment may vary in intensity, but it never induces the next segment to become active in a similar way.

Apart from their bearing upon the question of the segmental mechanism of the earthworm's locomotion, these data are likewise significant from the point of view of the physiology of its nervous system. The nervous system of the earthworm consists of cutaneous sensory cells and the ventral nerve-cord; sensory neurites pass from sensory cells to the cord and motor neurites, emerging from the latter, supply the muscles of the body. Golgi preparations of earthworm material show besides the neurites also dendritic processes, spreading out over the inner surface of the cutaneous epithelium and forming a sort of network. The function of this network is unknown, and although my experiments yield no final solution of this obscure question, the possiblilty that the network is an organ for the transmission of impulses is apparently excluded, since in none of the experiments was there ever an indication of a transmission of stimuli across a break in the ventral nerve-cord.

Studying the reactions of an earthworm with the nerve-cord interrupted in the region of the girdle, we find that moderate stimuli, both mechanical and electrical, when applied to the head start a contraction-wave running posteriorly, which ends abruptly as soon as it reaches the break in the nerve-cord. Applying a stimulus immediately anterior to the break in the nerve cord, a strong constriction of the body-wall is produced near the "nerveless"

portion, and a muscular contraction-wave runs forward therefrom. Strong stimuli may cause a more violent reaction, whereupon the worm will commence to creep and both parts, on either side of the inactive "nerveless" region, behave in a coördinated manner.

When, however, the stimulus is applied to the extreme posterior end, a much more vigorous reaction is generally obtained. The muscular contraction-wave, either starting at the most posterior point, stops abruptly at the "nerveless" part, or else, beginning immediately behind the "nerveless" area as a constriction of the body-wall runs backwards. But in no case does the impulse pass across the "nerveless" part.

From what has been said in the foregoing, it seems clear that muscular contractions in some part of the worm, unless very strong, are insufficient to induce a state of muscular activity in an adjacent part; and, as a matter of observation, locomotion consists of alternate lengthening and shortening of successive groups of segments.

Friedländer was surely aware of the fact that at times the earthworm behaves not as a series of individual segments but unmistakably as a unit. Such behavior, however, he believed to be peculiar of reaction to special stimuli, but not of ordinary quiet locomotion. "Es ist wahrscheinlich, dass der Regenwurm—und wahrscheinlich auch andere Anneliden—*nicht als einheitliche Individuen, sondern vielmehr sozusagen als Segmentreihen kröchen,* so lange keine besonderen Reize auf sie einwirken" (p. 202).

It will be my task in the following to demonstrate, that in quiet creeping, just as in the exceptional instances of strong stimulation, the principle of totality of the organism asserts itself very decidedly. This brings me to the consideration of the second problem, namely, what determines the earthworm's movements in a definite direction?

Although this problem has been studied extensively, the true nature of the worm's orientation in space must necessarily have remained an unknown quantity so long as the sole purpose of the investigations has been the discovery of the directive influence of stimuli. As far as I know, Jennings was the first to insist

that the worm may move in a given direction of its own accord, so to speak, *i.e.*, regardless of extraneous forces. Doubtless much of what I shall say has already been stated by Jennings ('06), in his excellent paper on the earthworm's movements, but the fact is not yet fully appreciated that the earthworn has an internal mechanism of precise orientation, whereby several modes of its behavior, hitherto unnoticed may now be explained. *The worm tends to move in a straight line and, even in spite of obstacles, it will follow in the chosen course with obstinate persistency.* If the head of a worm moving in a definite direction is pushed to the right or left side, it invariably returns to its former position, and the worm continues its straight course. If an obstacle, such as a lead block, is so placed across the worm's path that the worm, persisting in its course, must impinge upon the block at a right angle, the animal will retract its front portion and often back up some distance as soon as it comes in contact with the block. A few seconds later, however, it resumes the straight forward course only to bump once more against the resistance. This performance of intermittent bumping may last for some time, and yet the worm will persist in its course without swerving to the right or left side. But when the block is so situated that the angle of impact of the moving worm is less than 90° the animal either creeps alongside the block, or turning its head in searching movements until the obstacle is ultimately avoided, starts on a new course.

The tendency of the worm to move and to maintain itself pertinaciously in a definite direction has led me to inquire if there is present an internal mechanism of orientation which determines the course of the animal's movements at any particular moment. A simple apparatus was constructed for this purpose consisting of two plates of slate: a large plate firmly fixed to the support, and a small sector so pivoted to the first that it could be moved easily in a horizontal plane, as will be seen in the diagram (fig. 1) A pair of guards, also of slate, were at times attached on either side of the pivot in order to prevent the worm from swerving off at the moment of passing from one plate to the other, but this part of the apparatus was not essential and could generally be dis-

pensed with. During the experiments the entire surface of the apparatus was kept moist. The experiments were conducted in a dark room with a single electric lamp so arranged that the fixed plate was completely shaded.

In a general way the experiments were so conducted that a worm crawling upon the sector was directed so as to pass on to the immovable plate while maintaining a straight course; in the meantime the sector would be turned through an arc of 10° to 45° either to the right or to the left. The result of this shifting was that, while the anterior part of the worm remained in the line of its movement, the posterior part was more or less deflected

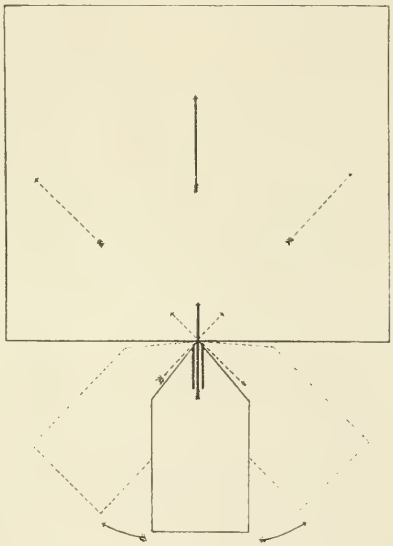

Fig. 1

from that line. Under these conditions the anterior part changed its position, swerving towards the side opposite to the turning of the sector, and the worm continued its movements in a new but approximately straight line. These reactions of orientation with no extraneous directing influence, schematically represented by means of arrows in the diagram, fig. 1, are precise and constant, so that, with the conditions properly adjusted, they may be repeated many times with scarcely a failure. It has been found

from a number of trials that worms neither too sluggish nor excessively agile and irritable are best adapted for the experiment.

The mode of reaction depends upon two circumstances; first the amount of turning of the sector; secondly, the particular moment at which the worm passes on to the fixed plate. If the sector is turned to the left as soon as the anterior quarter of the worm has gained the fixed plate, the worm comes to a stand-still instantaneously. The suspension of activity lasts a few seconds, in some exceptional instances, indeed, it lasted 45 seconds—then the head of the worm changes its position with a sudden jerk to the right, the entire worm being thus brought again into a straight line oblique to its original direction, and the animal starts moving in the new direction. If at the moment of the turning of the sector to one side the worm is already half-way across, it does not cease moving even for an instant, but, swerving its head to the opposite side and thus assuming an S-shaped form, it continues upon the new path. After a while the worm's body again becomes straight. Turning the tail to the right or to the left in the region of the posterior third causes but a slight reaction of orientation in the anterior part of the worm.

The intensity of the orientation of the head depends upon the amount of turning of the tail, being the greater the further the tail is swung. It should be observed, however, that the amount of turning of the head is always smaller than that of the tail. Furthermore, the turning of the tail through an arc of about 20,° while sufficient to cause a bending of the head in an opposite direction, would fail completely to produce an effect if only a small portion of the tail were turned.

To reiterate: the extent of the orientation-reaction of the head is *directly* proportional to the length of the posterior part of the worm deflected from the straight course; and, similarly, the degree of deflection necessary to occasion the orientation-reaction is *inversely* proportional to the length of the deflected tail.

It is possible, of course, to influence the direction of the worm's movements more than once, by turning the sector of the apparatus first to one side and then to the opposite side. The worm may thus be forced to maneuver in a tortuous course. If a worm

that is moving in a straight line is made to assume a new course by turning the sector to the left (see fig. 2, *b*), it can still be redirected in an opposite way by quickly turning the sector to the right (see fig. 2, *c*). If, before the worm has passed beyond the limits of the sector, the sector is once more turned to the left, a third reaction sometimes occurs at the head, which swerves back to the right. The last reaction, according to the rule given above, is generally weak, but that it is effective is shown by the fact that the worm changes its course again. As a result of these manipulations the worm creeps in a zigzag fashion, as shown in fig. 2, *d*, and seemingly without any orientation. Careful observation,

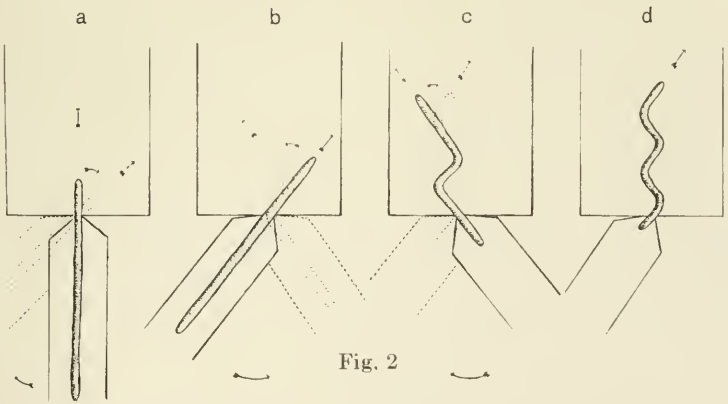

Fig. 2

however, reveals the fact that the worm persisting in the direction of the arrow, finally brings its body into a line coinciding with the course of its movements.

Returning now to the first problem set forth at the beginning of this paper—Does the worm in locomotion function as a unit or as an aggregate of independent components?—we may attempt to answer it on the basis of the facts just stated. Friedländer, defending his thesis that in locomotion the earthworm reacts as a succession of independent segments, was obliged to attribute reactions which obviously involve the entire organism to exceptional means of stimulation, thus restricting his analysis to normal quiet creeping alone. But the experiments on the spontaneous orientation of the worm show conclusively that also in quiet creeping the worm reacts not as a chain of segments but rather as

a unit. A change in the position of one part of the worm's body calls forth immediately a coördinated response in a remote part of the worm apparently due to an impulse passing from one end of the animal to the other.

Furthermore, these experiments show clearly that after some vacillation the worm turns in the direction which allows it most readily to maintain a straight course. The animal may turn towards or away from the source of stimulation, depending upon the relative position of its tail. If the animal is stimulated on the right side while its tail is bent to the left side, it recoils with a sudden jerk to the right, *i.e.*, towards the stimulus, until it has straightened itself; then it creeps away in the direction newly assumed. But if stimulated on the left side it also swings to the right, *i.e.*, this time away from the stimulus, and begins to creep when nearly in a straight position. Thus both reactions, towards and away from the source of stimulation, are but incidents of the more fundamental reaction of orientation. Other movements likewise, vaguely called random, may prove to be incidental to the spontaneous tendency of the worm to assume a straight course, and, therefore, in studying the earthworm's reactions to stimuli of low intensity this factor should be taken into consideration.

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EDITORIAL ANNOUNCEMENT

The Journal of Comparative Neurology, founded by the late Dr. C. L. Herrick, in 1891, was announced to include the morphology and physiology of the nervous system, together with studies in animal behavior and comparative psychology.

To insure a more adequate representation of the last two subjects, the Editorial Board was reorganized in 1904 with Dr. R. M. Yerkes as responsible editor for this department; subsequently Dr. Herbert S. Jennings and Dr. John B. Watson were associated with him.

The very rapid development of studies in behavior during recent years has produced a literature so voluminous and special that it has seemed best to these members of the Editorial Staff to organize, with the aid of other workers, a separate Journal of Animal Behavior, the first number of which is to appear early in 1911.

While it is a source of keen regret that this reorganization necessarily brings with it the severance of editorial relations which have been uniformly pleasant, the remaining editors desire to offer cordial greetings to the new Journal of Animal Behavior and to wish for it all success. They pledge themselves to cooperate in every way with this new venture, foreseeing that by its aid a still closer articulation of the study of the structure and functions of the nervous system may be attained.

This is the occasion to repeat the statement made in the original prospectus of The Journal of Comparative Neurology, that by comparative neurology is meant, not only the study of the nervous system in lower organisms, but all neurological researches carried on in the comparative spirit.

The editors therefore desire contributions dealing with the anatomy and with the physiology of the nervous system and sense organs of man and the lower animals; but recommend that papers in the fields of comparative psychology and behavior be submitted to the new journal, a more detailed prospectus of which follows this announcement.

In accordance with this change in scope, this journal, beginning with volume 21, will resume its original name:

THE JOURNAL OF COMPARATIVE NEUROLOGY.

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